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PROGRESS REPORT

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ENTOMOLOGY RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

CURRENT SERIAL RECORDS

Section B

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1967, and June 30, 1968. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D.C.

July 1, 1968



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AREA NO. 12 - INSECTS AFFECTING MAN, HOUSEHOLDS,  
AND INDUSTRIAL ESTABLISHMENTS

RPA 706 - CONTROL OF INSECT PESTS OF MAN AND HIS BELONGINGS

USDA and Cooperative Programs

Location of Intramural Work	Scientist Man-Years F.Y. 1968
	Research Problem Area 706
California	0.9
Florida	19.8
Louisiana	1.0
Mississippi	.3
Oregon	.4
Total	22.4

Intramural program is supplemented by extramural support representing (a) 2.8 SMY's at State Agricultural Experiment Stations, (b) 1.0 SMY's at other U. S. institutions, (c) 0.2 SMY's supported by AID, and (d) P. L. 480 funds in 1 country representing 110,613 U. S. dollars equivalents.

## Problems and Objectives

Insects and other arthropods affecting man, the household, and industrial establishments are responsible for heavy losses of food and materials. In addition, at least \$500 million is spent each year on household insect control and at least \$250 million on organized mosquito control. Many diseases resulting in loss of lives are transmitted by insects and other arthropods. Their annoyance results in reduced efficiency of labor, and discomfort in the home and recreation areas. Practical chemical control methods have been developed for many pests of man, but for some pests no control methods are known. Insecticide residues create hazards of contamination of food and the environment. Virtually no biological control agents that are effective have been found. Development of insect resistance to insecticides is a continuing threat.

Major objectives of the research are to develop and evaluate methods of insect control through:

1. Research on their biology and habits.
2. Improved insecticides and methods of use that minimize residue problems.
3. Attractants, repellents, and sterilization techniques.
4. Parasites, predators, and pathogens.
5. Integrated control including management of the environment.

## Progress - USDA and Cooperative Programs

### A. Basic Biology, Physiology, and Nutrition (4.7 SMY)

#### 1. Mosquitoes, sand flies, and gnats

At Gainesville, Fla., male Culex pipiens quinquefasciatus exposed to P<sup>32</sup> as larvae contained a high amount of radioactivity in their reproductive systems. About 31% of the total found in the reproductive organs was in the accessory glands (these glands occupied about 33% of the total volume of the reproductive system). About 0.09% of the radioactivity was transferred during insemination of a female. Individual males inseminated from 1 to 8 females for an average of 4.3 females over a 6-day period. There were variations in radioactivity among females inseminated by an individual male. Greater radioactivity of females caged with males 6 days as opposed to 3 days suggests multiple mating. Radioactive males averaging about 27,000 counts per minute compete very favorably with untreated males under laboratory conditions. Females exposed as larvae to P<sup>32</sup> deposited egg rafts that contained about 40% of the total radioactivity and the egg hatch was about 5% lower than in egg rafts from untreated females. In another study with males tagged with P<sup>32</sup>, 3 of 82 wild females collected and examined within 48 hrs after release of the tagged males showed radioactivity due to mating.

In further studies with this species 86 to 100% of the females and 63 to 94% of the males survived in 10 x 10 x 6-inch cages but in 4 x 8 x 6-foot cages, maximum survival of males or females was 68%. Sterilization by emerging through tepa-treated polystyrene strands had no harmful effect on survival. Aeration of the larval medium increased survival.

The rate of development between mid-September and mid-October in Florida was determined for Anopheles quadrimaculatus reproducing within an outdoor cage over 3 complete generations. The main difference between laboratory and field populations was the slower rate of completion outdoors of the third and fourth instars.

A culture of Aedes taeniorhynchus tissue was maintained for 32 days at the Gainesville, Fla., laboratory.

At the new, enlarged facility at Fresno, Calif., surveys indicated 8 species as the major problem species and 4 others that may also be important.

At Corvallis, Oreg., in studies on species composition and biology of the Culicoides gnat fauna of Benton County, a total of 17 species, of which 8 may be undescribed, were identified in the 1966 collections.

Indications were found at Corvallis, that Aedes dorsalis may be a facultative ecological homologue of Aedes melanimon. Mosquito larvae collected near Chemult, Oreg., appear to fit the description of Aedes communis nevadensis rather than typical A. communis communis. Orthopodomyia californica was found in Oregon for the first time. Culex tarsalis reared at 25°C were stronger and more fertile than those reared at 30°C.

At Lake Charles, La., study on the biology of salt marsh and rice field mosquitoes in screened and unscreened enclosures has continued for more than 3 years. Small numbers of salt-marsh mosquito larvae still appeared in two plots 3 years after screening. Eggs from early spring and late fall females of Aedes sollicitans, A. taeniorgynchus, Psorophora confinnis, and P. ferox showed a varied hatching response. Certain females produced egg batches in which some or all of the eggs were in a state of diapause. The trait was most pronounced in fall batches as opposed to spring batches. The trait was very evident in P. confinnis, less so in A. taeniorhynchus and present only to a minor degree in P. ferox and A. sollicitans. Eighty-eight water samples analyzed from tree holes above or adjacent to the ground that were producing mosquito larvae, showed that Orthopodomyia signifera, Toxorhynchites rutilus septentrionalis, Anopheles barberi and the non-biting midge Corethrella appendiculata were generally restricted to habitats characterized by high pH, very high levels of potassium, and high levels of carbonates and bicarbonates. Aedes triseriatus were usually found in habitats with chemical characteristics similar to but with lower concentrations than for these other species. Several other species were collected from habitats characterized by very low salinities. Soil samples analyzed from 30 breeding sites that produced A. sollicitans,

A. taeniorhynchus, and/or P. confinnis showed little or no difference in pH, carbonates, bicarbonates, or sulfates. However, the major difference between breeding sites of P. confinnis and the two Aedes was in chloride and sodium ion concentration. As the sodium and chloride ion concentrations decreased, breeding by P. confinnis increased. Nineteen oviposition preference tests showed that the three species exhibited definite preferences for concentrations of inorganic salts. P. confinnis showed a much stronger preference for salt-free sites. Sodium carbonate and bicarbonate, and, to a lesser extent, potassium chloride were detrimental to the eggs of all 3 species. Higher concentrations of the other salts were detrimental to eggs of P. confinnis. In oviposition preference studies with C. p. quinquefasciatus and C. salinarius, both species showed slight preference for sodium bicarbonate and sodium chloride as compared to sodium carbonate and sodium sulfate. Potassium chloride was preferred to sodium chloride, magnesium chloride and calcium chloride. Little or no preference was shown to a range of sodium sulfate concentrations. C. salinarius exhibited a broader tolerance for concentrations of sodium chloride, potassium chloride, calcium chloride, and magnesium chloride than quinquefasciatus.

Research has continued under two contracts at the University of Southwestern Louisiana and McNeese State College. Light trap studies indicated that the ratio of females to males for total catches was about 14 to 1. Anopheles crucians and Culex salinarius were collected the year around with the latter being the most abundant.

Light trap studies under a grant to Virginia Polytechnic Institute indicated that the two major species in that area were Culicoides sanguisuga and C. guttipennis. The former fed in significantly higher numbers at dusk, while the latter fed equally at dusk and in darkness. Under these experimental conditions, the site of the host--mammals and birds--is the major factor in attracting most of the collected species of Culicoides.

Research is continuing under a cooperative agreement with the University of Florida on the genetic basis of resistance to chemosterilants by mosquitoes. Considerable progress has been made in establishing a technique for analysis of suspected chromosomal aberrations in the apholate-resistant strain of Aedes aegypti, selected over 70 generations for resistance to apholate. Sterility in the strain has ranged between 30% and 50%; however, sterility amounts to 20% even when treatment with apholate is avoided.

## 2. House fly

Laboratory tests were conducted to select the most suitable non-fat dry milk for use in the diet of house flies at Gainesville, Fla. The milk used in the past had to be replaced every 2 days because it crusted badly and became unavailable to the flies as food. In a 7-day test, 24% of

the flies were dead with the old milk food and less than 1% of the flies died in the cages with the newly selected food. Preliminary tests were conducted in 2 x 2 x 2-foot indoor cages to determine the number of progeny that could be produced by house flies during their life span. The average number reaching pupal stage per female was 222.1.

At Corvallis, Oreg., tests confirmed that house fly resistance to pyrethrins was linked to hydrocarbon insecticide resistance in the kdr-0 strain. The tin-R gene confers resistance to organotin insecticides and intensified resistance to other insecticides by reducing absorption of the insecticides. Piperonyl butoxide synergized DDT in a strain for which resistance depends upon conversion to polar metabolites; the DDT analogue Dimite synergized DDT in a strain for which resistance depends upon the enzyme DDT-dehydrochlorinase.

### 3. Crickets

At Riverside, Calif., preliminary investigations indicate that breeding is extensive in alfalfa fields in the Coachella Valley and in a variety of field crops in Imperial Valley. The tendency of the soil to crack in Imperial Valley provides a favorable habitat.

### 4. Imported fire ant

Cooperative agreements to study the biology of the imported fire ant were negotiated with Mississippi State University, the University of Georgia, the University of Florida, and Louisiana State University.

Research continued under a PL-480 grant in Uruguay on biology of the imported fire ant. In mating, the males flew above the ground in a cone "circling" flight pattern; then left this cone to sieze a flying new queen and returned to the cone after mating. Female and attendant male fell to the ground where actual mating took place. After mating, the queen departed on an extended flight which became a migration or distribution flight. There has long been a question as to whether certain apterous females resembling queens could serve as queens. When 40 such females were placed in artificial nests with workers, all but one female oviposited and the workers cared for the eggs and ensuing larvae.

### B. Insecticidal and Sanitation Control (5.0 SMY)

#### 1. Mosquitoes

At Gainesville, Fla., the most effective materials screened against larvae of Anopheles quadrimaculatus were experimental compounds ENT-27464, Shell SD-9098, and ENT-27474-c-Gb which killed from 96% to 100% of the larvae at 0.01 ppm. of forty-three candidate insecticides evaluated as residues on plywood panels, 7 were effective. All 7 were effective for 24 or more

weeks, at a dosage of  $1 \text{ g/m}^2$  at half this dosage, only carbaryl, Banol, Shell SD-8530, and Geigy GS-13005 remained effective for the full 24-week duration of the test. In a new procedure, female quadrifasciatus mosquitoes from the susceptible colony and the Hartwell Dam (DDT and dieldrin resistant) colony were exposed to vapors from week-old residues on plywood panels. Against both resistant and non-resistant mosquitoes, only ENT-27039 was as effective as the standard Bay 39007.

Laboratory tests were conducted at Gainesville, to evaluate various insecticides as residues on flameproof cheesecloth at  $2 \text{ g/m}^2$  against A. quadrifasciatus adults. Bay 39007, carbaryl, DDT, Banol, Shell SD-8530, Mobam, and Geigy GS-13005 killed all the mosquitoes throughout the 88-week duration of the test. The malathion standard was 100% effective for 64 weeks. When Bay 39007, dye, and flameproofing materials (boric acid and borax) were applied to newspapers, the sprayed side remained effective for 68 weeks and the underside at least 76 weeks. On wrapping paper, the sprayed side was effective for 12 weeks, but the underside gave erratic results after 16 weeks. Burlap was dipped, rather than sprayed, and remained effective only 8 weeks; however, when Bay 39007 was added separately from flameproofing, the period of effectiveness increased to 20 weeks.

Field tests at Gainesville were conducted in a wall tent to evaluate Bay 39007 on polyethylene discs or Celotex strips and dichlorvos treated resin strips as fumigants for caged A. quadrifasciatus. With the treated materials suspended near the top of the tents, Bay 39007 was ineffective at the only dosage tested,  $2 \text{ g/10m}^3$  of tent volume. Dichlorvos was highly effective for about 2 weeks at  $2 \text{ g/10m}^3$ , but a week or less at 1 and  $0.5 \text{ g/10m}^3$ .

In practical field tests near Stuttgart, Ark., 9 insecticides were evaluated as residuals for control of A. quadrifasciatus in wooden buildings. Applied at  $2 \text{ g/m}^2$  to walls and ceilings, Bay 39007, Dursban, and experimental materials ENT-27213 and ENT-27334 caused 99-100% reduction of mosquitoes throughout the 10- to 12-week test. In another series of tests, yard-wide flameproofed cheesecloth treated with the insecticides was attached to the walls just below the ceiling. Dursban, Banol, Shell SD-8530, and ENT-27334 caused 95-100% reduction for 12 weeks; Mobam and Chevron RE-5353 were only slightly less effective.

Screening tests continued at Gainesville to evaluate candidate insecticides for control of Aedes taeniorhynchus, one of the salt-marsh mosquitoes. Twenty-two of the 70 tested had LC-90's less than 0.05% and 7 less than 0.02%.

Tests were conducted against caged Aedes taeniorhynchus adults at Gainesville comparing malathion fogs produced thermally (Leco 120) and chemically (interaction of ammonia and silicon tetrachloride). The

thermal fogs were at least 4.5 times as effective as the chemical fogs at distances of 900 feet, but both fogs were 100% effective up to 600 feet. Ultra-low volume aerosols were more effective than conventional high-volume aerosols against caged or free-flying salt-marsh mosquitoes when naled or malathion was the insecticide. Field tests were conducted in citrus groves with conventional aerial sprays for control of adult salt-marsh mosquitoes. Dursban was highly effective, giving 94% reduction at 0.025 lb/acre 6 hrs after treatment and 41% after 24 hrs. Bay 39007 gave 94% reduction at 0.05 lb/acre at 6 hrs, but no reduction after 24 hrs.

Tests were conducted in cooperation with the U. S. Army Forces Southern Command in Panama with thermal and non-thermal aerosols applied by ground equipment to control mosquitoes. Standard doses of malathion and fenthion did not give satisfactory kills due to dense vegetative cover. When 3 to 5 times the standard dosage was used, kill was adequate. In studies in cooperation with the Alaskan Air Command at Fairbanks, Alaska, malathion was about 7 times more effective than DDT when the LC-90's were compared. Ultra-low volume sprays of technical malathion were very effective. The dosage was 3.14 oz/acre, applied at 150 feet altitude by C-123 aircraft. An area of 15,000 acres was treated; control ranged from 94 to 96% for the first 3 days.

The relative tolerance of "non-diapausing" larvae of Mansonia perturbans to DDT and malathion was essentially the same as that found previously with "diapausing" larvae at Corvallis, Ore.

Under a contract with the University of California with low-volume applications, good mosquito control was obtained in rice fields and pastures treated by helicopter in the Colusa area, including the Colusa Wildlife Refuge. Dursban was applied at 0.05, 0.025, and 0.0125 lb/acre and fenthion at 0.1 lb/acre. The swath width was 250 feet and a volume of 6.1 fluid oz/acre was applied. So far, during studies of possible effects on bees and wildlife, no damage has been noted. In cooperation with the U. S. Navy and the California Bureau of Vector Control, studies were made in Kern County to determine whether low-volume applications could be successfully made at altitudes of 1,000 and 2,000 feet. Both larvae and adult mosquitoes were killed from altitudes up to 2,000 feet over swaths extending to 1/2 mile wide at dosage rates less than 8 fluid ounces per acre.

## 2. House fly

In laboratory spray tests at Gainesville, Fla., 18 candidate insecticides were equal to or more effective than the ronnel standard against susceptible and resistant house flies. In sugar bait tests, 15 out of 73 compounds tested were more effective than the trichlorfon standard. As residues on plywood panels, 8 of 43 compounds tested were effective for 24 weeks or more against both susceptible and resistant strains.

In wind-tunnel tests at Gainesville the F<sub>199</sub> generation of the Cradson-P colony (resistant) showed more than 12-fold resistance to parathion, more than 23- to 44-fold resistance to malathion, diazinon, and ronnel. DDT resistance could not be measured and is virtually complete. This colony also showed at least 2-fold cross resistance to all of the new materials tested in sugar baits except naled.

In field tests near Gainesville, Fla., against house flies, dimethoate residues were effective for at least 7 days and usually 9 to 11 days. Dimethoate, Gardona, and Bay 62863 remained effective as larvicides in manure under caged poultry for about a week, giving 75% mortality or better. Sixteen other materials tested were ineffective.

Under a cooperative agreement with the University of Florida, the continuous exposure test failed to detect resistance in the house fly to dimethoate and showed poor results with ronnel and fenthion while topical application demonstrated resistance to all three insecticides.

### 3. Stable fly

Research continued under contract with the Florida State Board of Health at Panama City, Fla., on the control of dog flies (stable flies). In tests with residual sprays on marine grasses, where much of the breeding occurs, DDT and methoxychlor were highly effective at 774 and 387 mg/ft<sup>2</sup> for more than 5 months; Bay 39007 at 100 mg/ft<sup>2</sup> was effective only 1 to 4 weeks. Up to 19 inches of rain had little effect on the residual effectiveness of DDT and methoxychlor. In tests with low volume aerial sprays, naled was much more promising than malathion.

### 4. Imported fire ant

At Gainesville, Fla., research is continuing on the development of bait toxicants for control of the imported fire ant, Solenopsis saevissima richteri. The addition of water glass (sodium silicate) to the attractant-mirex bait formulation increased the amount of oil that could be incorporated in the bait and also increased the retention of the oil. Baits treated in this manner are more effective and longer-lasting.

### 5. Body lice

In research at Gainesville, Fla., to develop more effective insecticides for the control of human body lice, 49 of 120 candidate materials screened were sufficiently promising for further testing. In secondary tests, 11 were 100% effective for more than 91 days as 1-percent powders.

Since the regular body louse colony now has some resistance to DDT, an attempt is being made to select a standard, non-resistant colony. Careful crossing of individual pairs yielded lice whose offspring showed increased

susceptibility. In the 7th filial generation, lice from the new colony were 36 times as susceptible at the LC-50 level and 15 times at the LC-90 level, as the regular colony. The Korean A colony (F225) has maintained its high resistance to DDT and the Freetown A colony its resistance to DDT and lindane (F148). After selection with carbaryl for 105 generations, lice of the Freetown C colony are practically immune to carbaryl and DDT, but are now slightly more susceptible to malathion than lice from the regular colony. Nine candidate insecticides were effective against resistant Korean A colony lice.

Three series of sleeve tests were conducted at Camp LeJeune, N. C., in cooperation with the Naval Medical Field Research Laboratory and Marine Corps personnel. No adverse medical effects were noted in any of the personnel wearing sleeves treated with 5% and 2% of Mobam or 2% of Abate; the compounds gave outstanding results, causing complete mortality of introduced lice for 6 weeks. World Health Organization's malathion-treated filter papers aged for 3 years still gave 100% kill of lice at the higher concentrations (0.8% to 12.8%) but the lower concentration (0.4%) showed a loss of effectiveness after 18 months and only 10% of the lice were killed after 3 years of storage.

#### 6. Ticks

At Gainesville, Fla., research continued on control of ticks affecting man. Tests were conducted at Camp LeJeune, N. C., in cooperation with the U. S. Naval Medical Field Research Laboratory to determine the effectiveness of Dursban, diazinon, Bay 41831, Bay 39007, fenthion, carbaryl, Shell SD-8530, and Mobam against natural infestations of lone star ticks. At a dosage of 1 lb/acre, all except carbaryl gave control averaging 90% or more, but the latter was effective at 2 lb/acre. Ultra-low volume application of fenthion gave excellent control with ground equipment (93% to 98% at 0.5 to 2 lb/acre) but aerial applications gave only fair control (68% at 1 lb/acre and 77% at 0.5 lb/acre).

#### 7. Bed bugs

At Gainesville 14 compounds tested caused at least 90% mortality of bed bugs in laboratory tests for at least 24 weeks. Laboratory tests showed that World Health Organization test papers impregnated with DDT or dieldrin and in storage for 66 months continued suitable for determining resistance levels.

#### 8. Cockroaches

In laboratory tests at Gainesville with contact sprays against normal (nonresistant) German cockroaches, 44 compounds were rated as Class 3 and 4, killing at least 75% of the roaches at 2% or at least 50% at 0.5% in the wind tunnel. Eight were highly effective as fresh residues on

plywood panels at 100 mg/ft<sup>2</sup>, but only 2 were effective for at least 4 weeks. In field tests, the average reduction of German cockroaches obtained in naturally infested buildings with 0.25% Dursban spray was 91% within 1 day and 97% within 2 weeks. Reduction averaged 98 to 99% during the 3 to 5 weeks after treatment, 96 and 95% after 6 and 7 weeks, respectively, and then fluctuated from 86 to 91% during the rest of the 12-week test period. Sprays containing 0.5% were more effective, producing a mortality of 100% for most of the 12-week test period.

Also in the laboratory, commercial formulations of Mobam dust were tested on plywood panels (500 mg of dust per square foot) against normal German roaches. A 5% Mobam dust produced 100% mortality throughout the 24-week test period. A 2% formulation produced 100% mortality for at least 3 weeks, and then 60 and 0% at 4 and 8 weeks, respectively. The 5% diazinon dust standard produced mortalities of 100, 75, 50, and 0% at 2 hr, 1, 2, and 3 weeks, respectively. Laboratory studies were conducted with boric acid powder applied to plywood panels against German cockroaches. The powder was highly effective provided exposure time was sufficient and post-exposure observation extended long enough to measure the slow ensuing mortality. For example, a 1,000 mg/ft<sup>2</sup> dosage with a 30 min exposure killed only 10% within 24 hrs and a 60 min exposure at this dosage killed only 25% within 24 hrs. However, even a 200 mg/ft<sup>2</sup> dosage with an exposure of 60 min killed 90 to 100% within 72 hours. This confirms the long-held view that boric acid and probably borax are effective, but slow-acting.

Studies on resistance of cockroaches to insecticides at Gainesville indicated that since 1955, with no selection by insecticides, colonies formerly showing 4-fold resistance to lindane, 8-fold resistance to dieldrin, and 18-fold resistance to chlordane, are now completely susceptible to these insecticides.

### C. Biological Control (4.6 SMY)

#### 1. Mosquitoes, sand flies, and gnats

At Gainesville, Fla., research on pathogens of mosquitoes has been continued. Five-hundred and ninety-two of about 1,000 adult female Culex, Aedes, Anopheles, and Psorophora species collected in central Florida were examined for pathogens. About 2.2% of ovipositing females were found infected with Microsporidia, 5.7% with Crithidia, and 4 specimens contained nematodes. All progeny of Microsporidia-infected females developed Thelohania infections; Anopheles crucians and A. quadrimaculatus were infected with Thelohania legeri and T. obesa. Of almost 100 adult female chironomid midges, 28 laid eggs but none contained Microsporidia. Nosema spores continued to infect the Gainesville colony larvae of A. quadrimaculatus, but attempts to transmit one species of Stempellia and 3 species of Thelohania failed to produce infections. Four adult female A. crucians collected near Micanopy, Fla., were infected

with Coelomomyces. Sporangia from this pathogen were transmitted to 2 larvae of A. quadrimaculatus. At least 2 species of nematodes were found infecting adult A. quadrimaculatus and A. crucians female mosquitoes in south central Florida. Plistophora species infections found in Psorophora ciliata were light, but those in Culex nigripalpus were heavy, causing gross disease symptoms.

Attempts at Gainesville to infect mosquitoes with Thelohania spores from field-collected larvae were unsuccessful. The colony Anopheles quadrimaculatus, wild caught quadrimaculatus, and also A. crucians and Culex salinarius sometimes contained spores that were distinct for each species. Furthermore, the spores in adults were distinct from those of their larvae. Larval progeny from adults infected with the smaller spores consistently developed Thelohania legeri infections; those harboring the larger spores developed T. obesa infections. The larval progeny of field-caught female C. salinarius developed a microsporidiosis identical to T. opacita. Adult females of the quadrimaculatus colony infected with T. legeri contained spores identical to those found in larval progeny of wild females of this species. The Nosema spores reported by other workers are concluded to be bimorphic forms of Thelohania. However, the Nosema species found in the quadrimaculatus-Nosema colony has produced high mortality (up to nearly 90%) and surviving adults are heavily infested. The culture in Anopheles is not a year old and the reason for increased virulence is unknown.

At Gainesville, Fla., electron microscope studies revealed that both types of Mosquito Iridescent Virus (MIV) represent a noninclusion virion with a dense central core, apparently icosahedra about 180 millimicra in diameter. Transmission studies with both the regular and blue types of virus in Aedes taeniorhynchus resulted in from 2 to 34% infection. The regular and blue MIV types do not differ in serological precipitation techniques and the reason for the color difference remains unknown. Attempts to transmit MIV to Aedes aegypti, Culex p. quinquefasciatus, and 5 species of moths were unsuccessful but a 1% infection was obtained in 7500 A. taeniorhynchus larvae released in a small pond supplied with salt water to simulate field conditions. In a new study, the MIV seems to be established in a tissue culture of Antherea eucalypti (a moth) furnished by the Insect Pathology Pioneering Research Laboratory, Beltsville, Md.

Mortality and a drastic decrease in egg production by adult Culex p. quinquefasciatus was due to Entomophthora coronata (determination verified by Insect Pathology Pioneering Research Laboratory and Crops Protection Research Branch, Crops Research Division, Beltsville, Md.). Since this fungus is known to be capable of infecting humans, female mice were exposed by topical, subcutaneous, intraperitoneal, and inhalation techniques. None died, all test wounds healed normally, and histological examination of mice injected with E. coronata from the mosquitoes showed

no evidence of infection. Attempts to transmit the fungus to house flies proved unsuccessful. With the cooperation of the Department of Veterinary Science, University of Florida, lesions were cut from the leg of a horse suffering from phycosis, supposedly caused by E. coronata. Cultures resulted in one isolate resembling Conidiobolus, three isolates of Rhizoctonia, one of Aspergillus, one of Actinomyces, and one of Histoplasma. Failure to isolate Entomophthera from a wound exposed to air and soil contaminants does not rule out Entomophthora as the causative agent. Another Entomophthora species was found in wild-caught female quadrifasciatus and an Achylya species isolated from larvae of Psorophora howardii.

At Corvallis, Oreg., microsporidiosis in Culiseta incidens collected from Philomath log ponds ranged from 0 to 80%. The infectivity of Thelohania campbelli was increased by the presence of certain bacteria in the medium in laboratory studies.

At Lake Charles, La., intensive research on mosquito pathogens as potential biological control agents continued. Patent infections of Thelohania were observed in larvae of 14 species of mosquitoes, and the non-biting competitive midge Corethrella brakeleyi. Peroral transmission of Thelohania inimita in Culiseta inornata was obtained on several occasions with screened plastic containers in the field. Stempellia magma was found in larvae of Culex restuans and Psorophora ferox; another Stempellia sp. was found in larvae of Toxorhynchites rutilus septentrionalis and the sand fly Culicoides nanus. Plistophora spp. were found infecting larvae of Orthopodomyia signifera, Culex territans, Culiseta inornata, and the sand flies Culicoides arbuticola, C. nanus, and in Corethrella brakeleyi. Plistophora caecorum was found infecting larvae of Culiseta inornata. In laboratory tests, Aedes aegypti appeared the most resistant to Plistophora culicis infection whereas Culiseta inornata was the most susceptible of 7 species of mosquitoes in 4 genera tested. The host was found to affect size variation in the spores of this protozoan.

Fungi infecting mosquitoes also continue to be studied at Lake Charles. Field infections of Coelomomyces spp. were found in 7 species. From 3 to 76% of the A. crucians populations in a pond near Chloe, La., were infected with Coelomomyces punctatus, with an average infection of 32%. Infections of this fungus were acquired by first-instar larvae of A. bradleyi, A. crucians, and A. quadrifasciatus in 24 of 49 screened plastic containers that were placed in the pond, but tests with Anopheles punctipennis were negative. Coelomomyces quadrangulatus was found infecting small numbers of larvae of A. crucians and A. quadrifasciatus. The basidiomycete commonly found in female Aedes sollicitans was isolated a number of times from a yellow exudate that is often seen on the salt-marsh grass Spartina spartinae.

In research at Lake Charles with mosquito iridescent virus (MIV), the virus was found in field collections of Aedes fulvus pallens, A. taeniorhynchus, A. vexans, and Psorophora ferox, and a possible infection in Aedes thibaulti. A single specimen of A. taeniorhynchus with the blue type of MIV was also collected. Additional infections with an iridescent virus (CuIV) were observed in larvae of Culicoides arbuticola in a tree hole at West Bay, La. Over 60% of the larvae were infected. Transmission per os was demonstrated with this virus in this species of sand fly. The level of infection of CuIV in Culicoides spp. from two tree holes ranged from 31 to 50% and averaged 37%. Forty serial passages of the regular MIV in A. taeniorhynchus to colony taeniorhynchus produced a mean transmission rate of 16%. Forty-one serial passages of the blue MIV produced a mean transmission rate of 17%. When females of taeniorhynchus were injected with regular MIV, about 45% of their progeny developed patent infections. When the females were permitted to feed on a 2% sucrose-MIV suspension, a small percentage of their larval progeny developed patent infections. Tests also showed that live, frankly infected larvae could release sufficient virus in a container to produce frank infections in uninfected larvae. Adult females were inoculated with the blue MIV and some of their larval progeny developed patent infections in the fourth instar. Adult taeniorhynchus in a cage were sprayed with MIV and the virus was transmitted transovarially to a small number of their larval progeny. When the survivors were permitted to pupate and emerge, the resulting adults produced larvae (F<sup>2</sup>) with a 38.4% level of infection. Transovarian transmission was demonstrated for the first time both with the blue MIV of taeniorhynchus to colony taeniorhynchus and the blue MIV of P. ferox to colony P. ferox.

At Lake Charles nematodes were found for the first time in Aedes atlanticus, A. mitchellae, Psorophora ciliata, P. discolor, and Uranotaenia lowii. Romanomermis nematodes were found infecting larvae of 12 species. This nematode exhibits very little host specificity and has been observed to penetrate and develop in 23 species of mosquito larvae, but not in Aedes triseriatus, Culex territans, or Psorophora ferox. Eggs of Romanomermis hatch in 7-10 days and the preparasitic juvenile enters the host larva through the cuticle. Maturation to the postparasitic stage takes 7-9 days; molting to adult, copulating, and laying eggs requires 11-15 days.

Romanomermis is now being maintained at Lake Charles, La., in the laboratory. It most readily invades the second instar of the laboratory host, Culex pipiens quinquefasciatus. About 34% of the parasitism took place in the first 5 hours of exposure. The nematode has little tolerance to salinity. Volume of water in the test container had little effect on the ultimate degree of infection. Gastromermis sp. nematodes were found in larvae of A. crucians and A. quadrimaculatus. The juveniles hatch 10-16 days after oviposition. Maturation to postparasitic stage takes 6-7 days after onset of parasitism.

The first mermithid nematode infection found in any Culicoides sand fly was in Culicoides nanus. Romanomermis nematodes were found in C. nanus and C. arbuticola. Nematodes of the family Tetradenematidae were recovered from larvae of C. arbuticola and the midge Corethrella brakeleyi.

The mermithid nematode, Agamomermis culicis, was found infecting about 20% of almost 5,000 female Aedes sollicitans collected from 24 different localities in Louisiana during warm weather, and 5% of 1256 adults from 9 different localities during cold weather. Over 95% of some adult sollicitans populations were infected; parasitism in individual collections ranged from 0 to 100%. This pathogen may be the prime factor in preventing outbreaks of sollicitans in Louisiana and Texas, during normal years.

At Riverside, Calif., under a research contract with the University of California, four isolants of Chrostoliphoris bacillus, each from different field collected mosquitoes, are being investigated. A large percentage of field populations is infected and mortality was high. A metabolite produced by an Aspergillus was found to be highly toxic to mosquito larvae. Purification and identification of this in additional metabolites is continuing, as are infectivity tests against other species of mosquitoes. Five isolates of field-collected green and blue-green algae produced metabolites toxic to mosquito larvae. Field observations that suggested a close correlation between the presence of certain algae and absence of mosquito breeding were confirmed by tests giving 98 to 100% mortality of third stage C. tarsalis and C. p. quinquefasciatus larvae placed in filtered water from pools containing the algae.

Research at Riverside continued under a grant to the University of California on the use of introduced annual fishes as a means of mosquito control. Nothobranchius guentheri eggs were found to survive through two 8-week drying periods, August to September, 1966 and May to July, 1967, with an intervening 7.5 months immersion in water at prolonged temperatures of 40° to 50°F (report on 1966-1967 studies). Hatch occurred after flooding in July 1967. Other eggs of N. guentheri developed while a pond was drained for only 11 days and hatched when the pond was reflooded. These findings indicate the species may be a much more flexible candidate for mosquito control in temporary breeding sites than formerly thought. The common guppy, Poecilia reticulata, may have possible use in polluted and temporary waters on an annual replacement basis. Laboratory tests indicated that 3-week old guppies would completely control the hatch from 200 mosquito eggs even in the presence of surplus alternate food.

In research under contract with McNeese State College at Lake Charles, La., on predators and parasites of mosquito larvae, most emphasis has been placed on three common minnows, Cyprinodon variegatus, Gambusia affinis, and Mollienisia latipinna. In laboratory tests to determine relative rate of mosquito larva consumption, Gambusia scored highest on basis of number of larvae eaten per fish per day and also on the ratio

of weight of larvae consumed per fish per day through the average body weight of fish tested. All three species fed actively enough on mosquito larvae to justify field experiments to test their potential usefulness.

## 2. House fly

At Gainesville, Fla., a fungus, Entomophthora muscae, from field-collected house flies from a poultry farm, produced high mortality in the laboratory. Addition of the pathogen to the standard larval rearing medium resulted in 100% kill of emerging flies. Histopathology studies showed that all body tissues were affected except the chorion of developing eggs. However, preliminary tests in screened cages showed no mortality of flies under field conditions. Laboratory tests conducted to determine the effect of Bacillus thuringiensis exotoxin showed high effectiveness against flies of both the regular, insecticide-susceptible strain and the resistant Cradson-P strain, when mixed with the larval medium.

## 3. Imported fire ant

Under a PL-480 grant in Uruguay progress is being made on methods of introducing Solenopsis (Labauchena) daguerrei into an imported fire ant colony with associated workers of the latter ant. Immobilization by cold (9-11°C) was found less hazardous than carbon dioxide anesthesia which killed some of the younger ants. A hypothesis on the mechanism of parasitization in the field has been advanced: Parasitization may be established when a daguerrei ant queen meets an imported fire ant founder queen in the process of founding a new colony.

## D. Insect Sterility and Other New Approaches to Control (4.7 SMY)

### 1. Mosquitoes

At Gainesville, Fla., 31 compounds that were effective as chemosterilants against house flies were screened for adult Culex pipiens quinquefasciatus. Nine of the chemicals caused complete sterility at 0.01% in sugar solutions fed on by the mosquitoes. Tepa caused complete sterility at the lowest concentration tested, 0.001%. Apholate, metepa, hempa, and porfiromycin sterilized at 0.05% and experimental compounds ENT-5055-a, ENT-51029, ENT-50787, and ENT-26398 at 0.1%. Males were consistently more sensitive than females to sterilizing and toxic effects of tepa and metepa. A 30-min exposure to tepa residues sterilized newly-emerged males, but 10-min exposures caused only partial sterility. Males caused to crawl through holes in styrofoam blocks treated with 5% tepa were completely sterile. Studies were also conducted to determine the sterilizing effects of gamma irradiation on males of this species. When day-old pupae were irradiated, 99.3% sterility was produced by 11 kr, and 99.6% by 12 kr.

In outdoor cages at Gainesville, Fla., about 50% of apholate-treated Culex p. quinquefasciatus males and Anopheles quadrimaculatus males died in the first 24 hrs, but only about 30% of the Culex females and 37% of the Anopheles females. Almost all surviving Culex females were inseminated within 48 hrs, but only a few of the Anopheles females were ever inseminated.

Studies on the sterile male release concept were conducted with C. p. quinquefasciatus in Gainesville. Males were sterilized with tepa or metepa and released daily into a population of mosquitoes breeding normally in a large outdoor cage. Estimates were made daily of the number of adult males and females emerging in the normal population and the number of sterile males released. Taking into account other studies that showed approximately 50% of the laboratory-reared sterile males died within the first 24 hrs, a theoretical per cent of the females which should lay sterile egg rafts was estimated. When sterile males were released in close proximity to the normal breeding area within the cage, the expected degree of sterility and the actual degree of sterility were practically the same; therefore, sterile males are able to compete sexually with the normal males in the insemination of virgin females.

Under a PL-480 grant to the University of Cairo, Egypt, a survey of the Wadi El-Natroun as a possible site for field studies on the control of mosquitoes by chemosterilants is continuing to determine mosquito density and the habits and behavior of the species found therein. Anopheles superpictus was collected for the first time in that locality. Laboratory studies with the chemosterilants tepa and hempa indicated that Culex pipiens could be sterilized with tepa but not with hempa.

## 2. House flies

At Gainesville, Fla., 61 of 575 candidate chemosterilants caused complete sterility in adult house flies. Untreated male flies exposed to topically chemosterilant-treated male or female flies became sterilized from 60 to 80% of the time. Field females were less receptive sexually to laboratory-reared sterile males than to field-collected fertile males. House flies forced to migrate through expanded polystyrene foam strands treated with 5% tepa were sterilized. Only 10 centimeters of depth was needed to accomplish sterility, even when an 18-cm depth was used. The male flies were completely competitive with untreated males, but 10 days after treatment there was 7% more mortality in the treated males. The maximum uptake of tepa recovered in flies that had migrated through the strands was 21.1  $\mu\text{g}$  for the integument, 24.9  $\mu\text{g}$  for the homogenate, and 57.8  $\mu\text{g}$  for environmental contamination (feces and vomitus) per fly. In a simulated field tests with flies sterilized by feeding on 1% metepa bait, metepa in the homogenate varied from 0.28  $\mu\text{g}$  to 5.64  $\mu\text{g}$  in males and 0.14  $\mu\text{g}$  to 15.0  $\mu\text{g}$  in females when a choice of diets was available. When only treated bait was present, metepa ranged from 0.54 to 6.38 in males and 0.36 to 15.2  $\mu\text{g}$  in females. Field tests with hempa bait disclosed that

captured flies contained 0.04  $\mu$ g to 4.67  $\mu$ g of hempa. In 24-hr old females treated with Squibb Olin 53330, the follicular cells were normal while in others vacuoles had formed around the nurse cell nuclei. The majority of the cells were vacuolated with clumped chromatin in those females 48 to 96 hours old and all cells of the entire follicle had degenerated, leaving only pycnotic remnants in the ovarioles of those 120 hrs old.

A chemosterilant bait containing 1% of hempa applied on droppings in a poultry house at half-weekly intervals at the rate of 5 g/m<sup>2</sup> produced a 75.0 to 93.6% reduction in the adult fly population for 9 weeks. Better results were prevented by migration of additional flies from a neighboring pig farm and stockyard. In another field test, the natural population of house flies was suppressed with insecticides before sterile flies were released. The released flies were resistant to the insecticide used and the insecticide treatment was repeated throughout the test. There was a marked reduction in flies and the ratio of released to wild flies increased from 3:31 to 6:1 with a total of 5 million flies released. This change in ratio was also accompanied by a gradual increase in the sterility of the wild population.

At Corvallis, Oreg., research on new methods of house fly control continued. A mathematical model was prepared that suggests that population control of the house fly could be obtained through use of conditional genetic lethals. A conditional lethal is one not lethal in the laboratory but lethal under field conditions. At least 17 such conditional lethals are being colonized at one laboratory or another in the World.

Under a PL-480 grant to the University of Cairo, Egypt, on the control of house flies by chemosterilants, field surveys in the Wadi El-Natroun area were initiated to determine changes in fly abundance and species composition. This is in preparation to a proposed field test of chemosterilants in an isolated area. Musca domestica vicina was the dominant species (78%) with peak populations in September and October and a minimum in January. Musca sorbens represented about 11% and other species 11%.

### 3. Body lice

At Gainesville, Fla., a synthetic juvenile hormone, ethyl farnesate, showed moderate toxicity to adult body lice (45% in 24-hr exposure to 1% solution on cloth). When female lice laid their eggs on freshly treated pads, only 7% of the eggs hatched, compared to 91% on untreated pads. However, the hormone does not seem to have practical value in louse control since eggs laid on the treated pad after the first day hatched normally.

### 4. Fleas

Research continued at Gainesville, Fla., on new methods of flea control through use of candidate systemic insecticides. Of 32 selected compounds

evaluated as oral systemics in guinea pigs; 5 materials showed promise at dosages ranging from 2.5 mg/kg to 400 mg/kg. The other compounds either were ineffective against the oriental rat fleas or killed the guinea pig. Experimental compound ENT-27462 at 200 mg/kg, Kepone at 100 mg/kg, and formothion at 200 mg/kg offered to rats without a choice of untreated food were ineffective. Trichlorfon at 400 mg/kg in bait to rats not offered a choice of untreated food was effective against oriental rat fleas for 14 days.

## 5. Tsetse flies

Under a PASA agreement with AID, research is continuing in Salisbury, Rhodesia in cooperation with the University College of Rhodesia on the feasibility of the application of sterile male technique for the control of tsetse flies. Productivity of the Glossina morsitans II heavyweight colony fell to a mean of 700 pupae per month due to the weakened condition of the host guinea pigs which underwent an epidemic of brucellosis. A third Glossina pallidipes colony was initiated and pupal production improved when the adults were held at  $25 + 1^{\circ}\text{C}$  from 0600 hrs to 1800 hrs and  $18 + 1^{\circ}\text{C}$  from 1800 hrs to 0600 hrs daily and were kept in darkness from 1200 hrs to 0800 hrs. However, even the increased reproductive capacity brought about by this regimen was still insufficient for sustained colonization and the colony was discontinued in the 29th week. G. morsitans survival was better under this regimen. Increased survival of G. pallidipes was obtained by reducing the adult cage size to one-half normal. Tests conducted in Bristol, England, with Dr. Nash's colony of G. morsitans suggested that "lop-eared" rabbits are good hosts. Pupal production per female fly was more than twice that obtained in Salisbury. Good results were also obtained with goats as hosts. Exposure to carbon dioxide for anesthetic purposes reduced the longevity of flies exposed when 2 or more days old, and temporarily inhibited feeding by freshly-emerged flies.

Ultraviolet light was attractive to adults of both morsitans and pallidipes under controlled conditions in the laboratory and under ambient conditions in a small outdoor cage between 1600 hrs and 0800 hrs. Under laboratory conditions it inhibited the normal negative response of morsitans males to high temperatures and high mortality resulted since the flies failed to retreat to available areas of optimum temperature. Indoors, blue light elicited a positive response from males and females of morsitans, red and white light were more attractive to females than males, and yellow light was least attractive. Glossina pallidipes response to blue or red light was erratic and yellow light was less effective. Blue and red light were ineffective in outdoor small cage tests. In field tests in the natural habitat of morsitans and pallidipes, ultraviolet light was ineffective. Laboratory and outdoor cage tests revealed no obvious selection or preference for various colored surfaces by either species.

Sterile males were released on the main island of the Sampakaruma group at about 3,800 per month for the first 4 months, 1,900 per month for the

next 3 months, and 900 per month during the eighth month. As a result, male collections remained high in the release area even when collections in the control areas were low. The percentage of females in the total collection dropped well below that of the control areas during the eighth month, suggesting that the release program may be effective.

## 6. Cockroaches

At Gainesville, Fla., laboratory studies were conducted with 3 selected chemosterilants to determine their potentialities with cockroaches. Nymphal German cockroaches were allowed to feed on standard laboratory chow treated with the chemosterilants and the effects on nymphal development observed. Adult specimens obtained from the feeding study were used in treated-treated and treated-untreated crosses to determine potential reproduction for either sex. None of the 3 chemicals produced complete sterility, but reduced hatch was obtained in all crosses of specimens fed diets containing 0.05% of experimental compound ENT-50909 or 0.5% of ENT-50910. Reduced hatch was also evident in the cross of treated sexes fed 0.05% ENT-50910. The productivity was normal for all F<sub>1</sub> generations in these studies.

## 7. Ticks

Research was initiated under PL-480 by contract with the Israel Institute for Biological Research at Ness-Ziona, Israel, on the development of sterility methods for population control of some soft ticks.

## E. Attractants and Repellents(3.4 SMY)

### 1. Mosquitoes and Gnats

At Gainesville, Fla., 40 new compounds were screened as repellents. Three were sufficiently effective for further testing. Experimental compound ENT-14913-GA and a mixture of this compound (75%) plus ENT-28633 (25%) were effective for 162 days. Allethrin and ENT-33423 were effective for more than 21 days. In rinse tests, ENT-14913-GA was effective after 30 min of rinsing. Of 210 compounds applied to 1/4-inch mesh cotton netting, 40 gave protection of 6 to 9 days or more. The most effective compound, ethyl propanediol, was effective for 221 and 205 days, respectively, against quadrимaculatus and taeniorhynchus. Laboratory tests were also conducted on 1/4-inch mesh cotton netting with the ingredients of M-1960 (the standard military clothing repellent impregnant). With the treated netting (0.5 g repellent per gram of netting) wrapped around the forearm, M-1960, ethyl propanediol, mixtures of benzyl benzoate and ethyl propanediol (1:1) and ethyl propanediol and N-butylacetanilide (1:1) gave protection of over 119 days against both Aedes taeniorhynchus and Anopheles quadrимaculatus. When M-1960 and deet were tested separately on 3/8-inch mesh netting, deet gave 53 days' protection against both species; M-1960 gave 46 days' protection against quadrимaculatus and 60 days' protection against taeniorhynchus.

Field tests were continued with 3 space repellents on netting at Camp LeJeune, N. C., in cooperation with the Naval Medical Field Research Laboratory and Marine Corps personnel. Nets (1/4-inch mesh) treated with 0.5 grams of M-1960 or 2-butyl-2-ethyl-1,3-propanediol per gram of netting did not give good protection from mosquitoes. Nets treated with this dosage of deet afforded fair protection of the test subjects. Heavy rainfall leached out the repellent.

Field tests near the east coast of Florida with space repellents on 1/4-inch mesh cotton netting and pressed cotton netting on booth-like enclosures were made natural populations of Aedes taeniorhynchus. Pressed netting treated with 5 chemicals and stored in a sealed jar for 257 days was still effective. The chemicals and number of days effective after storage were: ENT-3573, 167; ENT-2487, 109; ENT-11553, 109; ENT-28599, 109; and ENT-28598, 103 days.

Untreated cotton netting (knotted, 1/4-inch mesh) was field tested at Stuttgart, Ark., to determine if it would afford any protection from Anopheles quadrimaculatus mosquitoes. The test showed that the mosquitoes would go through the 1/4-inch mesh to get to a ram in the shed, but in two out of three tests only 25% as many entered the net-covered shed as when the opening was not covered.

At the request of the Department of the Army, six fabrics were tested for protection from bites by Aedes aegypti and Anopheles quadrimaculatus. North Vietnamese olive-colored material gave complete protection against quadrimaculatus and allowed an average of 0.75 bites by aegypti. Byrd cloth allowed an average of 0.25 bites of the two species. Cotton twill was less effective and North Vietnamese black material much less effective. Nylon hammock material afforded little or no protection and cotton poplin tropical material allowed more than 9.5 bites of both species.

In laboratory tests at Gainesville, Fla., designed to distinguish between olfactory and tactile repellency, ENT-773, ENT-1654, ENT-6498, and dimethyl carbate showed outstanding repellency in both types of tests. Dimethyl phthalate was outstanding in olfactory but not in contact tests. Deet, ENT-373, ENT-17586, and ENT-20297 were outstanding tactile but not olfactory repellents. Ethyl hexanediol, benzyl benzoate, undecenoic acid and three other materials were not outstanding in either type of test.

Deet, the outstanding repellent discovered through USDA research, has been found in extracts of female pink bollworm moths, where it is reported to play a role in attracting male moths. Deet and an extract of female moths gave comparable results when used to impregnate clothing. Results of such a comparison were also similar in skin tests, confirming biologically the chemical findings that deet is present in the female moths.

At Gainesville 104 chemicals were screened in an olfactometer as candidate attractants for Aedes aegypti. Twenty-four showed some promise. L-Lactic

acid seems to be the substance that attracts mosquitoes to man. Persons having the largest amounts of lactic acid on their skin were the persons most attractive to mosquitoes. This break-through culminates more than 10 years' research to determine how man attracts mosquitoes.

Seventy-two compounds were tested as repellents in spot tests on cattle at Corvallis, Oreg., against Aedes aegypti in the laboratory and in the field against A. dorsalis. Only three of the compounds provided repellency: ENT-28740, ENT-33516, and ENT-33518. They gave excellent repellency for 2 days at 1,000 mg/ft<sup>2</sup> but little or none at lower dosages.

Studies continued at Corvallis on the attractiveness of log pond waters to ovipositing Culex pipiens quinquefasciatus. The previously-tested chloroform extracts proved to be attractive even after evaporation to dryness; however, when the amount of dry extract increased above the equivalent of 20 microliters of chloroform extract, the attractiveness decreased. At the equivalent of 80 µl the attractiveness was the same as distilled water and at the equivalent of 160 µl attractiveness was less than distilled water.

Research under a grant to the University of Florida on attractants for the eye gnat, Hippelates, showed the highest population density in July. Over 95% of the gnats collected were females. An unexpected finding from soil samples collected from 10 different sites was the emergence of the gnats from two samples obtained from cattle holding pens. Tests to determine if adult gnats could be marked by dusting pupae with fluorescent dyes indicated that the method had promise, as all emerging gnats carried a detectable amount of dye for more than 28 days. Laboratory-reared gnats mated readily with wild populations and viable eggs were obtained in such crosses.

In research under a PL-480 grant to the Israel Institute for Biological Research, a method for comparing the effectiveness of repellents on cloth as determined by oviposition of Aedes aegypti was developed. Gravid females had to withstand the effects of a repellent-treated cloth at various periods after treatment in order to reach an oviposition site. The two most effective repellents tested thus far were deet and dimethyl phthalate.

## 2. House fly

At Gainesville, Fla., over 450 cyanides, thiocyanates, and isothiocyanates were tested as arrestants. About 10% of the cyanides were more effective than the sucrose standard. The best was N,N-bis(2-cyanoethyl)-m-toluamide. Tests with over 50 toluamide and benzamide derivatives suggested that the cyanoethyl moiety may be responsible for the arrestant activity of this material. Three of a series of straight-chain aliphatic nitriles--acetonitrile, propionitrile, and butyronitrile--were strong arrestants,

with propionitrile the best. Activity decreased abruptly with chain lengths greater than 4 carbon atoms.

At Corvallis, Oreg., research was continued on the sex pheromone reported earlier in the house fly. Efforts to purify, characterize, and identify the attractive principle in extracts of female flies have continued. Fourteen samples prepared by the Pesticide Chemicals Research Branch were evaluated, bringing the total number of extract-derived samples tested up to 270. Benzene extracts have never shown strike responses on the part of the male flies. Tests were made to determine whether the benzene extracts might contain masking agents to prevent strike responses. No evidence was found in two series of tests.

### 3. Deer flies

At Gainesville, Fla., research on development of repellents for deer flies continued. Three types of untreated head nets were tested concurrently with skin repellents against deer flies, primarily Chrysops atlanticus at Richmond Hill, Ga. The deer flies did not hesitate to land on 32-mesh khaki-colored nylon net; some landed on 3/8-inch white cotton net, but no flies entered the net. Very few landed on a 1-inch white cotton net; occasionally one would enter, but none bit. The cotton nets were more comfortable to wear than the nylon and easier to see through, but were more readily tangled in the underbrush than the nylon net. In a round-robin design series of tests, no significant difference in repellency was shown between 15% deet and 11% deet plus 4% MGK 874. None was shown in the paired tests comparing 75% deet and 75% deet plus 5% MGK 874.

### 4. Chigger mites

Research continued at Gainesville on the development of repellents for control of chiggers. One of 15 compounds screened for possible usefulness gave a complete knockdown in less than 1 minute and withstood 5 hot soapy water washes. This material (ENT-27334) and carbaryl were field tested as sleeve treatments in comparison with the benzyl benzoate standard. Both a water emulsion and an acetone solution of ENT-27334 withstood 2 washes; the water treatment of carbaryl withstood one wash and the acetone treatment was ineffective after one wash. Benzyl benzoate remained effective after washing.

In cooperative studies with the U. S. Naval Medical Field Research Laboratory at Camp LeJeune, N. C., three chigger repellents were tested as clothing treatments on Marine Corps utility uniforms. Eleven uniforms were treated with benzyl benzoate (standard), or M-1960, or carbaryl. All gave 100% protection after the first week of wear but after the first laundering, and the second week of wear, 2 out of 11 treated with M-1960 and 2 with benzyl benzoate failed. After an additional laundering and the third week of wear, 4 more with M-1960, 3 more with benzyl

benzoate, and 1 with carbaryl had failed. Thirty days after treatment with three launderings, 2 treated with M-1960, 3 with benzyl benzoate, and 3 with carbaryl remained effective. The odor of benzyl benzoate was objectionable and carbaryl seemed to bleach the uniforms. M-1960 treated uniforms felt damp and oily. Carbaryl did not depress cholinesterase of those wearing it in a uniform.

#### 5. Ticks

At Gainesville, Fla., research continued on the development of repellents for protection from ticks. Six single compounds and 10 mixtures of deet and another chemical were tested in wooded areas infested with nymphs and adults of the lone star tick near Richmond Hill, Ga. Army fatigue uniforms were treated with the single compounds at 2 g/ft<sup>2</sup> or the mixtures (equal amounts combined) at 3.2 g/ft<sup>2</sup>. The two most effective tick repellents were mixtures of ENT-12191 and deet with a protection of 5 weeks and one of ENT-5550 and deet which gave a protection period of 4 weeks.

#### 6. Fleas

Research continued under a PL-480 grant to the Israel Institute for Biological Research at Ness-Ziona, Israel, on the action of repellents against fleas. Fifty repellents were evaluated against the oriental rat flea on guinea pigs. Four were about equal to the standard, deet.

#### 7. Yellow jackets

At Corvallis, Oreg., 86 synthetic compounds were screened as attractants for yellow jackets, but none was attractive.

Research on yellow jacket lures was initiated at Fresno, Calif. No report is yet available on exposure of synthetic lures to workers, but queens were caught in Malaise traps and carbon dioxide traps.

#### 8. Cockroaches

At Gainesville, Fla., laboratory tests were conducted to evaluate the effectiveness of selected chemicals as attractants for German cockroaches. Of a total of 1,256 selected compounds tested, only 10 attracted 5% or more roaches. The most effective compound, ENT-28479, attracted 9.25% of a group of 250 adult cockroaches in the test. This was a slightly greater attraction than that shown by Coca-Cola syrup (9%).

#### 9. Body louse

At Gainesville research was initiated on possible olfactory attraction of body lice. It was noted that male and female lice respond to human

odor by walking toward the point of release. Carbon dioxide was also attractive, but less so. Lice starved 24 hours before testing responded better, but a 48-hour starvation period left the lice too weak for consistent results and 8 hours after feeding, there was no response.

#### 10. Triatoma infestans

A relative of the "Mexican bed bug", Triatoma infestans, is relatively large (about 1-inch long) and has large neurons that are comparatively easy to isolate, making this insect essentially ideal for study of olfactory neuron response in a blood-sucking insect. At Gainesville, Fla., interesting relationships on the qualitative aspects of odor perception have been shown. Two main types of neurons have been identified; both are sensitive to human breath, furan, and pyridine, but not to carbon dioxide, warmth, deet, dimethyl phthalate, or oil of citronella. Neurons of one type show a low spontaneous activity and a phasic burst of action potentials during stimulation. The second type shows relatively high spontaneous activity that is reduced or inhibited during stimulation. Since each neuron preparation may remain stable over a period of at least 2 weeks, the spectrum of sensitivity to many chemicals can be ascertained with this insect.

#### Publications - USDA and Cooperation Program

##### RPA 706 - CONTROL OF INSECT PESTS OF MAN AND HIS BELONGINGS

#### Basic Biology, Physiology, and Nutrition

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AREA NO. 13 - BEES AND OTHER POLLINATING INSECTS  
(RPA-314)

USDA and Cooperative Program

Location of Intramural Work	Scientist
	Man-years FY 1968
Maryland (Beltsville)	1.0
Arizona	6.0
Louisiana	5.0
Louisiana	1.0
Utah	3.0
Wisconsin	1.0
Wyoming	1.0
Total	18.0

Intramural program is supplemented by extramural support representing  
(a) 0.8 SMY's at State Agricultural Stations and (b) 0.7 SMY's at other  
U.S. institutions.

## Problems and Objectives

Honey bees are the most important of all the insect pollinators. Their pollinating activities perform a necessary service in the production of fruit crops, and vegetable and legume seed crops. This pollinating service is performed incidental to foraging for foods for their own use (nectar and pollen). Through beekeeping, pollinators are distributed throughout all the cultivated areas. This makes available a manageable supply of pollinators for use where and when they are required. This self-supporting pollination service is provided mainly through the production and sale of honey and beeswax. Therefore, to maintain an adequate level of these pollinators, beekeeping must be kept in a profitable condition.

Major objectives of the research are to develop and evaluate alternative ways to:

1. Obtain maximum utilization of bees in crop pollination.
2. Increase productiveness of bees through genetics and breeding.
3. Develop more efficient colony management practices.
4. Develop methods for better control of bee diseases and pests.

## Progress - USDA and Cooperative Programs

### A. Biology and Breeding for Improvement of the Honey Bee (5.0 SMY)

At Baton Rouge, La., a total of 44 queens were produced from an original stock of one queen selected in a pollen study in New Jersey in 1966. Twenty-five were tested in New Jersey and discontinued.

At Madison, Wisc., two colonies were selected for "high" cranberry pollen collection and two for "low." Daughters of these queens will be used for further breeding and selection for "high" and "low" cranberry pollen collection.

At Baton Rouge, La., a bioassay for gibberellins in royal jelly showed that extracts from royal jelly placed on plants in 20  $\mu$ l doses in either acetone or ethyl acetate damaged the leaves and possibly inhibited growth. Smears of royal jelly also caused damage to the bean leaves, but the onset of damage to the leaves was more gradual.

At Baton Rouge, La., five groups of bees were set up for alfalfa pollen collection tests. Matings were completed at Baton Rouge and selection tests were conducted in the area of Logan, Utah.

In general, results were similar to those obtained in previous years. A table summarizing relative performance of the groups of colonies tested follows:

Group	% APC collectors	Number APC collectors	Number pollen collectors	Number nectar collectors	Honey yield per colony
High inbred	86	29	34	97	27
Low inbred	8	4	46	89	29
High virgin outcross	52	18	35	102	44
High drone outcross	59	18	30	130	82
Commercial	31	16	49	115	50

At Logan, Utah, it was noticed that alfalfa pollen pellets from the trap on the high APC lines tended to be larger than those from the commercial or low line colonies. There were 133 pellets per gram in the high and 191 per gram in the commercial. This indicates that a higher percentage of the bees bringing back alfalfa pollen among the high APC colonies were "true pollen collectors." Small pellets generally result from the activities of the so-called nectar-trippers -- bees primarily seeking nectar but often using the direct approach to the flowers.

At Baton Rouge topical application of a juvenile hormone on the thoraces of 25 workers from 5 nuclei and 4 colonies that were queenless, showed no effect on ovarian development. Bee samples taken from the brood area of four queenless colonies revealed that ovarian development occurs in the second and third week of queenlessness. Laying workers appeared in the third and fourth weeks.

#### B. Management for Improvement in Productivity of Honey Bees (1.0 SMY)

At Madison, Wisc., "Heger supplement" (a soybean-meal-terramycin product) appeared toxic to honey bee brood, since treated colonies removed all their young brood after treatment with the product.

At Madison, Wisc., seven synthetic pollen substitutes formulated by the Research and Development Division of the National Dairy Products -- Kraft Division gave marked increase in brood rearing over check colonies

but the best synthetic material supported only 248 square inches of sealed brood as compared with 360 square inches supported by our standard 25% pollen-75% soybean flour supplement.

At Madison studies of the economics of various methods of colony management, including package, two-queen, and single-queen management, showed that the efficiency in terms of honey production per unit of time with the two-queen system is superior to all other systems.

At Madison forty queens were held in a special colony from November 4 until March 31 in 2" x 2" cages containing comb and faced with zinc queen excluder material. They were all laying eggs simultaneously in January and brood developed normally.

At Madison, treated binder twine used as smoker fuel had a toxic effect on honey bees. After 10 smoke treatments on caged bees in a 2-week period, 89% of the bees were dead from binder twine smoke, as compared with 8% of the untreated check bees.

At Madison a modification of blower equipment, using a heavy-duty air compressor with a venturi nozzle to obtain greater air volume, was used successfully for blowing bees from honey supers. This equipment was displayed at the XXIst International Apicultural Congress.

At Beltsville, Md., caged bees were fed 33, 50 and 60% sugar sirups and dry sugar. The higher the sugar concentrations, the more water the bees required. Bees died within 10 days when fed only capped honey. When fed dry sugar and no water, the bees survived for 4 days. Bees survived only 2 days when water alone was placed in the cages.

#### C. Etiology of Bee Diseases and Development of Control Methods for Diseases and Pests (3.0 SMY)

1. Nosema Disease. At Laramie, Wyo., individual bees were inoculated with nosema spores by pipette feeding. After one week, during which the caged, incubated bees were provided plain sugar sirup, 100% of the inoculated bees sampled for dissection contained spores of Nosema apis in their midintestines. Then the food provided some of the cages was changed to sirup containing 5 grams of Fumidil-B per gallon. In succeeding weeks the relative numbers of spores in the midintestines decreased markedly, and appeared entirely eliminated in some bees, but increased in bees continuously fed plain sirup. Moreover, some bees dissected during the third week of feeding fumagillin-sirup had no spores in their midintestines, but still had spores in their rectal contents, indicating that their midintestines originally had been infected but that fumagillin prevented further infection of their midgut epithelium,

the spores being gradually eliminated in their feces. Therefore, fumagillin enables individual honey bees to actually recover from an initial Nosema infection.

At Laramie six chemotherapeutic materials were fed to caged honey bees for control of Nosema disease. Furacin (nitrofurazone) proved highly toxic at 200 ppm. None of the six materials tested prevented Nosema infection, although there was some indication that paromomycin at 200 ppm may have inhibited the development of the parasite to some extent. Other materials tested and found ineffective in preventing Nosema infections were: monensin, plaquenil, trimethoprim, and Burroughs Wellcome Co.'s "Compound 356c61."

At Laramie in two series of replicated cage tests 12 additional chemotherapeutic agents, at two different dosages, were compared with fumagillin for their ability to prevent Nosema disease in artificially inoculated honey bees. Hygromycin proved highly toxic to bees at both 200 and 20 ppm, anthelmynin was highly toxic at the higher dosage only and vibramycin and kanamycin at 200 ppm were somewhat toxic. None of these, or the other chemicals tested (daraprim, flagyl, rondomycin, alvosulfon, eucalyptus oil oxodene, primaquine, or urotropin) were effective in preventing Nosema infections. Fumagillin at a dosage of 20 ppm reduced Nosema infections by about 80%.

At Laramie *Nosema apis* spores held at about -10°F for 30 days, either in whole bees or in bee midintestines in 0.6% saline solution, were still highly infective, producing nearly 100% infections within one week in caged healthy bees.

At Laramie live healthy honey bees kept 30 minutes in a deep-freeze at about -10°F did not fall to the bottom of their cages, but bees infected with Nosema fell down when held at this temperature for 15 minutes. At the 15-minute exposure both infected and healthy bees recovered, and most lived for 2 weeks.

At Baton Rouge combs from twenty-four colonies, divided into six groups of four each, were treated with ethylene oxide and acetic acid, and the bees fed sugar syrup and Fumidil B. Data of the amount of Nosema infection indicated that those treated with an ethylene oxide-Fumidil combination and those with acetic acid-Fumidil combination were not significantly different from the control colonies.

At Baton Rouge *Nosema apis* infected bees were found to contain larger percentages of saturated fatty acids and a higher percentage of fatty acids of chain length 16:0 and below. When compared to noninfected individuals, the infected samples showed decreases in the relative percentages of oleic and stearic acids with increases in palmitic, linoleic, and linolenic acids.

At Beltsville, Md., a temperature of 120°F was effective in virtually eliminating Nosema apis from honey combs.

2. American Foulbrood. At Laramie, Wyo., combs contaminated with American foulbrood (AFB) were fumigated within a black plastic sheet three times at a dosage of 2 pounds of "Oxyfume" containing 12% ethylene oxide at each treatment. Treated and untreated AFB combs were given to healthy colonies in an isolated apiary. One colony given treated AFB scales developed slight disease in 10 to 38 days. Colonies given treated ropy AFB, bee-bread, honey, or empty dark combs remained healthy. Control colonies given untreated AFB scales, ropy material, or bee-bread developed moderate to severe disease; one colony given untreated empty dark combs developed a trace of disease; one colony given untreated honey reared only a small amount of brood in adjacent combs and did not develop disease in late autumn. The recurrence of AFB in ethylene oxide combs treated at low temperatures (outdoor temperatures below 70°F), indicates the need to control temperatures in order to obtain sterilization.

In experiments on the control of American foulbrood using plastic enclosures, at Beltsville, Md., it was found that the degree of success could be improved by two introductions of ethylene oxide instead of one. Levels of 1 or 2 pounds used twice yielded the most satisfactory results. By using ethylene oxide it was possible to recover 27 of 28 colonies that had American foulbrood.

3. European Foulbrood. At Laramie, Wyo., two colonies which developed severe natural infections of European foulbrood, and in the same apiary a third colony in which EFB was suspected because of extremely spotted brood, were gorged 3 to 5 times with 2 grams tylosin per gallon of 50% sugar sirup. The weakest colony was also requeened. All 3 colonies appeared to eliminate this disease and develop uniform, healthy brood.

At Laramie the "high alfalfa pollen collecting" strain of honey bees appears to be highly susceptible to European foulbrood, since colonies of this strain developed severe natural infections of EFB, while other strains in the same apiary showed light or no infections.

A severe natural infection of European foulbrood in 1 colony in late June at Laramie was greatly reduced by gorging the bees with sugar sirup containing tylosin, and the infection was gradually eliminated by the bees.

At Beltsville, Md., ethylene oxide was effective in reducing the incidence of European foulbrood. In tests conducted in New Jersey, one treatment with 2 pounds of Oxyfume-12 was as effective as 3 dustings with Terramycin (TM-10). At the end of the season, the control colonies had 8.7 frames of

bees, the TM-10 colonies had 13.2, and the ethylene oxide colonies 16. The increase in frames of bees indicates the effectiveness of the treatment. Likewise, the larger colonies should be more effective as pollinators, nectar gatherers, and be better able to overwinter.

4. Wax Moth. At Laramie, Wyo., a small hymenopterous parasite found emerging in a laboratory culture of the greater wax moth, Galleria mellonella (L.), was identified as the braconid, Apanteles galleriae Wilk.

At Beltsville, Md., all stages of the greater wax moth were killed when exposed to 120°F for 2 hours. A temperature of 110°F failed to kill all stages of the wax moth, even after a 24-hour exposure period.

5. Amoeba Disease. At Laramie, Wyo., attempts to infect caged honey bees with amoeba cysts failed at both low and high temperatures (75 and 92°F.), even when the bees were known to have ingested the cysts by individual feeding by pipette. A natural amoeba infection in a single colony affected primarily the oldest bees.

6. Sacbrood. At Laramie the Baton Rouge "standard" strain of honey bee larvae, inoculated individually with the same inoculum at the same age, appeared to be more susceptible to sacbrood than either the high or low alfalfa pollen collecting strains. However, results were complicated by natural infections of European foulbrood, and by partial drone laying queens, so the comparisons were discontinued.

7. Drug Toxicity. At Laramie chlorine dioxide at dosages of 10,000 or 1,000 ppm in 50% sugar sirup, produced 100% mortality in caged honey bees within 1 to 3 weeks. Lower dosages permitted increased longevity of caged bees. Mortalities at the end of 11 weeks averaged 50% in the cages fed 10 ppm chlorine dioxide, and 66% and 78% in the cages fed 100 ppm or 1 ppm, respectively, compared to 79% mortality in the control cages fed plain sugar sirup.

8. Ethylene Oxide Toxicity. At Laramie a double treatment of combs with ethylene oxide gas, using approximately 2 pounds of "Oxyfume" containing 12% ethylene oxide per standard hive-body at each treatment, inside a sealed metal tank with the pressure attaining a maximum of 15 psi, temperatures ranging from 60° to 101°F and the combs being ventilated at 88°F for 24 hours before they were given to nuclei, did not cause any abnormal mortality

compared to an untreated control. There were no toxic residues in sealed or unsealed honey, in bee-bread, or beeswax, under the conditions of this test.

D. Behavior and Utilization of Honey Bees in the Pollination of Agricultural and Other Economic Crops (7.0 SMY)

At Tucson, Ariz., drivert, a commercial product of cane sugar plus 10% invert sugar, was found to have some advantages in making up candy bee-feed. However, limited tests with it as an ingredient in pollen candy revealed difficulties in achieving a suitable texture.

At Tucson several methods of extracting phagostimulants from pollens revealed that different pollens may require different extraction techniques.

At Tucson for the second time field-scale efforts to stimulate pollen collection by feeding sugar sirup were unsuccessful.

At Tucson radioactive carbon ( $C^{14}$ ) was fed in glucose to bees in order to elucidate information on the synthesis of amino acids.  $C^{14}$  was incorporated measurably in glutamic acid, proline, alanine, and lysine. It was detected, but not quantitated, in aspartic acid, aerine, and threonine.

At Tucson limited tests with the Woods Mark 5 Apidictor revealed that it diagnosed normal colony conditions reliably, but was not able to diagnose wax moth infestation or robbing.

Techniques for obtaining bee hemolymph were compared at Tucson in preparation for obtaining cholesterol data. Pipette collection of the droplet of blood oozing from the thoraces of decapitated, eviserated bees was confirmed as the best method. The volume of hemolymph was found to be extremely variable.

At Tucson thirty-two honey bee colonies of varying population sizes were established for a 24-day period in 4-foot-cube boxes. At the end of this time comb structure, design, and placement were examined for clues to factors affecting dimensions of bee combs. The analysis of variance between the three comb dimensions of height, width, and length showed no difference in these dimensions when all 32 colonies are considered as a group. However, since significant differences did appear in two of the four population groups, it is possible that the influence of these three dimensions are not exactly equal. Comb built by queenless bees is different from any described in the literature. The occurrence of multiple clusters building comb with and without queens in the same box was recorded but unexplained. Measurements of cell size gave the following number of cells per square decimeter: worker, 813.8; drone, 540.0; queenless, 678.3.

At Tucson pollen supplement containing three levels of pollen fed to nuclei in cages of alfalfa permitted continued brood rearing. Unfed control nucs reared no brood. Amount of brood seemed related more to amount of food consumed than to proportion of pollen.

At Tucson four small "nuc" colonies were maintained in cages under a plastic "tent" in the laboratory. The "tent" was erected to maintain humidity close to 50%. The bees were given water and sugar sirup in plastic bags inside the hive as well as pollen supplements containing 20 to 25% pollen. The bees have reared brood and have drawn out foundation. Six similar colonies set up in an outside cage are also doing well.

At Tucson total hemocyte numbers per  $\text{mm}^3$  of hemolymph are higher in worker bees from a colony maintained at 8500 feet elevation than in bees from a colony at 2360 feet. Differential hemocyte counts of queen larval, pupal, and adult hemolymph are essentially the same as obtained from workers and drones. Protein concentrations in hemolymph were found to be highest in pupal hemolymph and lowest in adult hemolymph. Electrophoretic analysis demonstrated distinct differences in protein bands in the hemolymph of brood of various ages and in the adults of the 3 castes. Human serum albumin (HSA) (5 microliters of 1% solution) was injected into bees. Differential hemocyte counts were no different in injected and control bees, but the HSA-injected had more total blood cells than the control bees. HSA-injected bees also showed differences in electrophoretic protein band patterns.

At Tucson "stopping" response of bees to substrate vibration peaked between 500 and 600 Hz. If continued, strong colonies adapted and resumed activity while weak colonies tended to abscond. Foraging activity appeared to be inhibited during vibration.

At Tucson, Ariz., the sterol fractions of pollens from 15 species of plants in 11 families were analyzed by mass spectrometry. 24-methylene-cholesterol was the principal sterol of red clover (T. pratense), saguaro cactus (C. gigantea), mustard (B. migra), London-rocket (S. irio), rye (S. cereale), timothy (P. pratense), and sweet corn (Z. mays var. sacch.). B-sitosterol was the principal sterol of mule fat (B. viminea), juniper (J. utahensis), heartsease (Polygonum sp.), waterleaf (H. capitatum), scotch pine (P. sylvestris), European alder (A. glutinosa), and lombardy poplar (P. nigra italica). Cholesterol was the principal sterol of cottonwood (P. fremonti) pollen.

At Tucson, Ariz., saline alone had no harmful effects when "inseminated" into a virgin queen. Tepa plus saline injected into oviducts of queens had no detrimental effect on the oviduct. Effectiveness of sterilization was a function of tepa concentration.

At Tucson square steel-frame hives were established containing comb area equivalent to 3.8 standard Langstroth hive bodies. Bees did well in them.

Production records were kept and several manipulation methods worked out which required turning the hives upside down to simulate hive-body reversal. Queen cells turned upside down were destroyed only if they were unsealed or just sealed. Bees utilized worker cells for honey storage and brood-rearing combs turned 90° and 180°.

Study of pollen samples in the Warrens, Wisconsin, cranberry section showed pollen collected from 14 plant families. Most abundant plant competitors in the cranberry area are Cyperaceae and Leguminosae and to a lesser degree Polygonaceae, Compositae, and Rosaceae.

At Madison, Wisc., the production of pickling cucumbers in cages with and without bees showed that bees greatly increase yield (33 pounds of fruit with no bees compared with 137 to 182 pounds where bees are used). The quality of fruit is also greatly improved where bees are used. In open fields the value of the cucumber crop was three times greater where bees were used as compared with fields without honey bee colonies.

At Madison the feasibility of using disposable cardboard package pollinating units of bees with a virgin queen treated with carbon dioxide to induce egg laying was demonstrated.

At Baton Rouge, La., it was found that packages of bees headed by CO<sub>2</sub> treated virgin queens had almost the same activity as those headed by normal queens. Virgin queen headed packages reared drone brood and appeared to maintain themselves for over six weeks.

Cranberry pollen has been suspected as a poor pollen source. However, based on tests at Beltsville, Md., using longevity and hypopharyngeal gland development, cranberry and blueberry pollen were superior to dandelion.

At Tucson, Ariz., of 50 queens tested, 4 of the 10 selected for highest cotton-flower-visitation and 2 of the lowest CFV were sent to Baton Rouge. These were used for crossing, and the offspring will be tested in 1968.

E. Effect of Pesticides, Insect Diseases, and Farm Practices on Honey Bees and other Pollinating Insects (1.0 SMY)

At Madison, Wisc., one application of carbaryl (Sevin) spray on September 4 to a 55-acre field of canning sweet corn located 1/2 mile from an apiary maintaining valuable breeder queens in holding colonies resulted in a loss of two thirds of the queens and a weakening of the surviving colonies, adversely affected their overwintering. Corn pollen brought into the hives two days after spraying contained 7.62 ppm carbaryl and 0.092 ppm free naphthol.

At Tucson, Ariz., using a new method of dosing insects with a hand-held microsyringe three insecticides were tested in acetone by topical application. Their LD-50 toxicities in µg/bee were: carbaryl 0.2 to 2.0; parathion

.04 to .08; Azodrin .02. Three antidotes were tested. When fed in sugar syrup, atropine sulfate was slightly toxic at 1%, P2S was safe at 1%, and PAM killed at 0.1%. Antidotes were ineffective when supplied in sugar solutions or in drinking water. Topical applications of 8 µg atropine plus 80 µg P2S failed to save bees treated with 2 ug carbaryl or 0.2 µg crotonamide. A slight effect was observed with antidotes applied in acetone. When atropine was injected, 4 µg/bee was toxic and 2 µg with or without 20 µg P2S gave little reduction in kill with crotonamide, carbaryl, or parathion.

At Tucson four normal bee colonies and one food-depleted were exposed to two and three applications of Azodrine at 1.5 pt/acre. Knockdown occurred in 15 minutes with aerial sprays; topical applications in the laboratory gave knockdown in 150 minutes. Nectar and pollen collected by bees from sprayed fields was not toxic when fed to worker bees or used to rear brood. Covering with burlap or polyethylene was ineffective in protecting bees. Present indications are that single applications applied before active foraging in the morning have only a slight effect on the colony.

At Tucson, Ariz., the infrared absorption reflected through the epicuticle of drone wings shows no ester groups expected of waxes. The functional groups that would accompany the presence of other lipids are also undetectable. Bees are not expected to have a cement layer covering the epicuticle. Nevertheless, the observed reflection spectra resemble that of pleated-sheet polyamides. This suggests that the surface of drone wings is mostly a protein similar to silk, wool, hair, or human skin.

At Tucson, Ariz., densitometer quantitation of the proteins of honey bee hemolymph confirmed earlier conclusions and gave a permanent record of protein separation. Polyacrylamide gel electrophoresis gave better separation of blood protein than did cellulose acetate electrophoresis of adult worker hemolymph. Attempts to immunize rabbits with bee hemolymph are underway.

At Tucson, Ariz., beehives were modified to allow honey bees to enter and leave the hives with either ingressing or egressing air. One test showed a highly significant separation with more bees entering the hive where the air was egressing. Significantly more bees also left the hive through the entrance where air was ingressing.

At Tucson, Ariz., rapid movement of air across hive entrances was effective in keeping bees from entering or leaving their hive. Velocities of 1500-1700 feet per minute were required to prevent departure, but velocities of 1100-1300 fpm were adequate to prevent reentry.

#### F. Biology and Utilization of Insects other than Honey Bees in the Pollination of Agricultural Crops (1.0 SMY)

At Logan, Utah, it was found that Union Carbide UC-21149 used at 0.1 gm of

10% granular material per soil pot ( a #10 can) gave excellent control of aphids and several other harmful insects but also killed most of the foraging wild bees when they visited the flowers. In February 1968, lower dosages of granular UC-21149 was applied to plants infested with green peach aphids and visited by nesting alfalfa leaf-cutting bees. Control of the aphids for 10 days was achieved with 0.012 gm of 10% UC-21149 per can at which dosage no apparent damage to leaf-cutting bees resulted. Even 0.003 gm of the UC-21149 held the aphids in check but did not eliminate them.

At Logan, Utah, four species of alfalfa pollinators were received as cocoons from Iran. These were Anthidium florentinum, Megachile rufipes, Megachile terminata, and Megachile sp.

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Area No. 14. ANALYSIS, SYNTHESIS, FORMULATION, AND  
EVALUATION OF INSECT CONTROL CHEMICALS

USDA and Cooperative Program

Location of	Scientist Man-Years F.Y. 1968										Total
Intramural	Research Problem Area										:
Work	: 204	: 207	: 210	: 314	: 315	: 701	: 706	: 901	: 906	: 201	:
Maryland	: 8.1	: 7.7	: 1.2	: 0.2	: 0.7	: 3.5	: 13.5	: 0.9	: 1.4	: 6.2	: 43.4
(Beltsville)	:	:	:	:	:	:	:	:	:	:	:
Florida	:	:	:	:	:	:	: 2.0	:	:	:	: 2.0
Georgia	:	:	:	:	:	: 0.4	:	: 0.2	:	:	: 0.6
Mississippi	:	: 3.0	:	:	:	:	:	:	:	:	: 3.0
Texas	:	:	: 0.1	:	: 1.8	: 1.1	:	: 0.3	:	:	: 3.3
Washington	: 0.5	:	:	:	:	: 1.0	:	: 0.3	:	:	: 1.8
	:	:	:	:	:	:	:	:	:	:	:
Total	: 8.6	: 10.7	: 1.3	: 0.2	: 2.5	: 6.0	: 15.5	: 1.7	: 1.4	: 6.2	: 54.1
	:	:	:	:	:	:	:	:	:	:	:

Intramural program is supplemented by extramural support under P.L. 480 funds in 1 country representing 73,618 U. S. dollar equivalents.

### Problems and Objectives.

The success of modern agriculture in producing an abundance of high-quality food and fiber depends in large measure on the use of chemical agents to kill insects which prey on crops and livestock. The problems attending the use of toxic materials fall in 2 general categories: 1) loss of effectiveness through development of insect resistance and 2) potential hazard to other creatures, including man, from the application or from the residual contamination of food and environment.

Therefore the major objectives of research are to find safer and more effective agents for insect control by:

1. Investigation of products of natural origin.
2. Synthesis of new types of chemicals which exert control through effects other than death.
3. Formulation of mixtures using synergists or carriers for specific conditions.
4. Biological evaluation of effectiveness of new materials through initial testing in the laboratory and final performance in the field.
5. Development of new analytical methods for the accurate determination of toxic residues and breakdown products in treated crops and animals and in the natural environment.

### Progress - USDA and Cooperative Programs

#### MULTIPLE RPAs

#### A. Products of Natural Origin as Sources of Insect Control Materials (15.2 SMY)

1. Insect sex attractants. Research continues at Beltsville, Md., on the isolation and identification of the sex attractants of various important insect species. In cooperation with the Canada Department of Agriculture valeric acid was identified as the sex attractant of the female sugarbeet wireworm. Although this is chemically the simplest natural sex attractant yet discovered, the identification was made from only 18 insects. (204, 207)

Under a research contract Midwest Research Institute synthesized a number of compounds needed in larger amounts for sex attractant studies. (204, 207)

A highly active mixture of isomers based on the natural juvenile hormone structure has been synthesized by two different routes as the result of an intensive team effort. (204)

At Yakima, Wash., an extract of 20,000 virgin female codling moths yielded only 7 micrograms of pure sex pheromone. This was enough material to establish that a significant chemical feature was the presence of an epoxy group. On the basis of this knowledge a series of epoxy compounds was synthesized for bioassay at Yakima, Wash. Twenty of the 21 synthetics evoked a sexual response from male moths and the most potent of these was cis-9,10-epoxy-1-octadecanol, active in test solutions at a concentration of 1 part per billion. (204)

Specifications were prepared for the purchase of cabbage looper sex attractant for testing in the field. (204)

The presence of N,N-diethyl-m-toluamide (deet) in female pupae and adults of the pink bollworm has been confirmed. It appears to activate propylure, the sex attractant, but its significance is not fully understood. The material is not detected in the male. (207)

Approximately 8 million insects and 55 kg of fecal material have been processed at State College, Miss., to isolate the sex attractant of the boll weevil. A compound active in laboratory bioassays has been separated and is in the initial stages of identification. (207)

At Gainesville, Fla., L-lactic acid was identified as the major attractive component in human odor for the female Aedes aegypti mosquito. The relative attractiveness of individual persons to the mosquitoes corresponded to the quantity of L-lactic acid found in acetone washes of their hands. (706)

Male American cockroaches exhibit a strong sex response to an extract of the feces of virgin female roaches 10 days to 3 weeks old. (706)

The first insect extract to show activity against the 5 WM tumor in tests at the National Cancer Institute was a fraction prepared by reducing the combined acids from female gypsy moth abdominal tips. The ketoalcohol believed to be responsible is being confirmed by synthesis. (201)

2. Materials of plant origin. Chemists at Beltsville have isolated, purified, and are in the process of identifying a sesquiterpene from Angelica seed oil which equals trimedlure in attractancy to Mediterranean fruit flies. (204)

All-trans-echinacein (N-isobutyl-2,6,8,10-dodecatetraenamide), an insecticidal constituent of American coneflower (Echinacea angustifolia), has been synthesized. (207)

At State College, Miss., the polar lipids of Delta Pine smoothleaf cotton buds were separated and determined. In general the percentages of fatty acids followed the pattern in other kinds of plants. An exception is a greater proportion of di- as compared to tri-unsaturated C18 acids in the phosphatidyl ethanolamine fraction. The variations of polar lipid concentrations in glanded and glandless lines of cotton buds are being studied as indicators of feeding stimulant activity. (207)

A study was made of the content of 6-MBOA (6-methoxy-2-benzoxazolinone) in Stowells' Evergreen corn. Since the compound is a factor in resistance to larvae of the European corn borer, leaves and kernels were analyzed at various stages of development. It was found that the amount of 6-MBOA declines sharply in corn leaves, from 6950 ppm at 15 days to 63 ppm at 90 days; in kernels, the decrease was from 435 ppm at 5 days to 4 ppm at 30 days. (207)

Under a P.L. 480 grant to researchers in India several chemical fractions from leaves and fruits of Melia azedarach and Melia indica (neem) have shown considerable repellency to face flies in tests at Beltsville. Pure materials isolated from these fractions include norriterpenoids, xanthophylls, and sterols, which will also be tested as insecticides. (210)

B. Development of Synthetic Organic Chemicals for Insect Control.  
(18.1 SMY)

1. Preparation of synthetic organic compounds for testing as insect chemosterilants. C<sup>14</sup>-labeled N,N,N',N'-tetramethylmelamine was synthesized at Beltsville for metabolic studies in insects (see E.2). (706)

Also prepared was the N-(hydroxymethyl) derivative of pempa (pentamethylphosphoric triamide), the latter being the major metabolite found in house flies treated with hempa. Tests showed this synthetic to be as active as hempa as a male fly chemosterilant. It is almost certainly an intermediate in the metabolic path from hempa to pempa despite the fact that it has not been detected in previous studies. The compound appears to be the first synthetic N-(hydroxymethyl)phosphoramidate. (706)

The synthesis of boron-containing chemosterilants was completed. Available results indicate that activity is largely restricted to female insects and that the active agent is boric acid resulting from in vivo hydrolysis of the compounds. (706)

A number of new aziridiny compounds were prepared for chemosterilant testing under a grant to the University of Pennsylvania. (706)

Midwest Research Institute prepared under contract a number of compounds used as test materials or as intermediates for synthesis by chemists at Beltsville. (207, 706)

Various toxicity tests with tepa and hempa were conducted by Hazleton Laboratories, Inc., as contract research. These included 2-generation reproduction and 90-day incubation studies in rats and dermal studies in rabbits. All were completed except the 2-generation reproductive study of hempa in rats which is still in progress. (701, 901)

2. Preparation of synthetic organic compounds for testing as insecticides, insect attractants or repellents, or synergists. Synthetic cis-7-hexadecen-1-ol acetate prepared at Beltsville as a homolog of propylure, the natural sex attractant of the pink bollworm, has proved to be a powerful attractant for male pink bollworm moths in the field. One pound of the compound has been ordered by commercial synthesis for field tests on a larger scale by the Cotton Insects Research Branch. The tentative name is "hexalure" and a patent application for its use as a survey and control agent for the pink bollworm awaits results from the above field tests. (207)

Another homolog produced at Beltsville in the same synthetic program is trans-7-decen-1-ol. This compound and the acetate derivative attract female Mediterranean fruit flies but not the males. Additional amounts of the acetate have been submitted for testing. (204)

Midwest Research Institute provided moderate amounts of several compounds for field-testing as attractants and repellents. (207, 706)

At Gainesville, 6670 formulations were prepared for testing against insects affecting man. (706)

C. Aerosols for Insect Control. (0.3 SMY)

At Beltsville a new plastic one-shot aerosol valve was tested for greenhouse use. The actuator was found to be about 1/1000 inch too long for reliable performance. When this defect was corrected by the manufacturer the valve was deemed satisfactory for its purpose. (204, 906)

D. Biological Evaluation of Chemicals for Insect Control. (4.3 SMY)

1. Insecticides. Through the central facility at Beltsville a total of 352 compounds from various industrial or government sources were distributed to laboratories of the Entomology Research Division for preliminary evaluation as insecticides or acaricides. Of these 159 were new materials. (See also E.2) Many of these compounds were also submitted cooperatively for evaluation by the Stored Products Insects Branch, Market Quality Research Division, at Savannah, Ga. (Multiple RPAs)

In addition, 120 materials from 33 industrial sources and the Pesticide Chemicals Research Branch were further evaluated in the field. Several of these materials showed promise as broad spectrum insecticides with a favorable margin of toxicity to warm-blooded animals. (Multiple RPAs)

Tests at the Beltsville laboratory show that face flies selected through exposure to residues of Ciodrin over 18 months tend to resistance after the sixteenth generation. (210)

After less than 1 hour of exposure to an 8-week-old residue of (5-benzyl-3-furyl)methyl chrysanthemumate, mortality of chlordane-resistant German cockroaches was 100%. (706)

## 2. Materials that control insects through effects other than death.

New noninsecticidal materials distributed for preliminary evaluation to selected laboratories of the Entomology Research Division included 442 samples derived from natural products and 581 synthetic chemicals prepared by the chemists at Beltsville. Approximately 640 more compounds were acquired from outside sources, mainly private industry or other government agencies. A little over half of these were submitted primarily for tests as attractants or repellents and about 40% of them as chemosterilants. (Multiple RPAs)

Metabolic studies with the chemosterilant hemel (hexamethylmelamine) were continued at Beltsville. Four metabolites of hemel also detected in treated male house flies and their feces had the following order of sterilizing potency: pentamethylmelamine > hemel > N,N,N',N'-tetramethylmelamine > N,N,N'-trimethylmelamine > N,N',N'-trimethylmelamine. More active as a sterilant than any of the above is N,N,N',N'-tetramethylmelamine which was not detected as a metabolite in flies or feces. Thus the metabolic pattern has a marked similarity to the demethylation of hempa. Postulated is an active intermediate formed within the insect during the demethylation of each parent compound. (706)

In an attempt to identify the above hypothetical intermediates it was found that in vitro demethylation of hempa proceeds with the evolution of formaldehyde. Indications are that the process also occurs in vivo although the intermediates were not isolated. A method developed to determine pempa (pentamethylphosphoric triamide) in the presence of large amounts of hempa can be used to show that in vitro demethylation of hempa is linear with time at 30° C. (706)

Another approach to the fate of hempa in house flies was a comparative study of the metabolism of C<sup>14</sup>-labeled hempa in normal flies and in flies resistant to Isolan (a dimethylcarbamate ester). Although hempa was equally effective as a sterilant to male flies of both strains, the Isolan-resistant flies degraded it at a much faster rate. (706)

In vitro studies with microsomal preparations from flies resistant to the carbamates Isolan and Bay 39007 showed that demethylation of hempa to pempa was several times more rapid than in nonresistant strains. Analysis of these extracts from flies revealed the presence of small amounts of N,N,N',N'-tetramethylphosphoric triamide which were proportional to the period of incubation but never more than 5% of the amount of pempa. The tetramethyl compound was not detected in extracts of the nonresistant strain. (706)

In a cooperative experiment with the Apiculture Research Branch in Tucson, Ariz., bee sperm was treated in vitro with tepa and used to inseminate virgin queens. A concentration of 25 ng of tepa per 3  $\mu$ l of sperm produced nearly 100% mortality of zygotes. (314)

At Gainesville, Fla., a study on the respiration of ovarian mitochondria isolated from the house fly revealed that the respiratory rate varies with the stage of ovarian development and the substrate used. In general, the rate is highest at early vitellogenesis. Respiratory control could be demonstrated only at certain stages of development and was unrelated to the respiration rate; it was also independent of exogenous inorganic phosphate. The respiration rate could be increased by the administration of metepa in vivo and/or tepa in vitro and the greatest stimulation occurred with mitochondria isolated from ovaries during early stages of development. (706)

Commercial samples of terpenoid materials were bioassayed at State College, Miss., for attractancy to boll weevils. Despite some inconsistency in the responses, some materials appeared to equal the standard extract of the cotton plant in attraction. (207)

3. Aerosols and space sprays. In tests against resistant house flies at Beltsville a new synergist Niagara NIA-16338 (propyl 2-propynyl phenylphosphonate) proved more effective than piperonyl butoxide in an aerosol formulation with Neopynamin (2,2-dimethyl-3-(2-methylpropenyl)cyclopropane=carboxylic acid ester with N-(hydroxymethyl)-1-cyclohexene-1,2-dicarboximide). The new formula (G-1662) was equal to the G-1357 Federal Specification aerosol in practical dosage tests against resistant house flies and Aedes aegypti mosquitoes. (706)

It has been necessary to find a substitute for Deobase (deodorized kerosene base) in sprays and aerosols since it is no longer being produced. A similar product, Dispersol, gave good results as the replacement for Deobase in the G-1357 Federal Specification aerosol against resistant house flies. It was also satisfactory in the Official Test Insecticide against susceptible German cockroaches. (706)

Another material no longer available is Velsicol AR-50 which has been the solvent for pyrethrins and sulfoxide in the Federal Aerosol Specification. An aerosol containing 3% Panasol AN-2 and 10% petroleum distillate was as effective against resistant house flies as the Federal Specification Aerosol containing 5% Velsicol AR-50 and 8% petroleum distillate. (706)

E. Methods for Control of Insects in Aircraft. (0.5 SMY)

Several species of insects collected in the field at Beltsville and representing the major orders (Orthoptera, Coleoptera, Lepidoptera and Diptera) were exposed to an atmosphere high in carbon dioxide. All insects so treated for 4 hours revived within several hours. Insects exposed for 48 hours did not recover. (Multiple RPAs)

RPA 315 - IMPROVEMENT OF GENERAL PURPOSE FARM SUPPLIES

A. Formulations. (2.5 SMY)

A representative from Beltsville was a member of the working party of the Food and Agriculture Organization of the United Nations on official control of pesticides to develop international specifications for pesticide formulations. Specifications were accepted for parathion, dieldrin, sulfur, sodium chlorate, nicotine, and DNOC. Tentatively accepted were specifications for various formulations of malathion, 2,4,5-T, a BHC-DDT mixture, lindane-DDT mixtures, toxaphene, toxaphene-DDT mixtures, toxaphene-DDT-methyl parathion mixtures, and carbaryl as well as draft specifications for certain formulations of diazinon, thiram, nabam, zineb, and lime sulfur. (315)

RPA 701 - INSURE FOOD PRODUCTS FREE FROM TOXIC RESIDUES  
FROM AGRICULTURAL SOURCES

A. Methods of Analysis for Insecticide Residues. (7.7 SMY)

At Tifton, Ga., a dual-flame photometric detector for gas chromatography was fabricated and tested. The device permits simultaneous analyses for phosphorus- and sulfur-containing compounds with high sensitivity. With it analytical methods were developed for residues of 3 important insecticides in field crops. (701)

In an investigation of residues in corn and grass treated with an emulsion spray of fenthion at 0.5 pound per acre, it was found that a high proportion of the fenthion was converted to metabolites almost immediately. Of 5 metabolites detected, the principal product was fenthion sulfoxide. All 6 compounds were found on grass, 5 on corn. For as much as 21 days after application, 5 persisted on grass, 3 on corn. (701)

A similar method developed for corn treated with granular phorate revealed a like pattern of rapid conversion to the sulfoxide. Small amounts of 4 other metabolites were also detected in various parts of the treated corn plant. (701)

When the method was adopted for residues of disulfoton and 5 of its metabolites, neither disulfoton nor its oxygen analog could be detected in field-treated tobacco. Residues were mainly disulfoton sulfoxide and sulfone plus small amounts of the sulfoxide and sulfone of the oxygen analog. (701)

Chemists at Yakima, Wash., and Beltsville, Md., have modified an analytical method previously developed for Union Carbide UC-21149 and its toxic metabolites so that time of analysis is cut by about one-half. Residues are all converted to the sulfone of UC-21149 for measurement. The method has been applied to alfalfa, apples, cottonseed, cucumber, and potatoes with a sensitivity of 7 parts per billion. (701)

Also developed was a gas chromatographic procedure for determining Abate (O,O-dimethyl phosphorothioate O,O-diester with 4,4'-thiodiphenol) in larvicide suspensions and residues of the compound in mosquito larvae treated by injection of the suspension. For the method it was necessary to design a cooling adapter for the flame photometric detector to permit operation at high temperatures. Sensitivity is about 2 ng of Abate in 5  $\mu$ l of injection suspension. (701)

Publications - USDA and Cooperative Programs

MULTIPLE RPAs

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Area No. 15. IDENTIFICATION OF INSECTS AND RELATED ARTHROPODS.

USDA and Cooperative Program

	:	Scientist Man-Years FY 1968								:								
Location of	:	Research Problem Area								:	Total							
Intramural Work	:	204	:	207	:	210	:	112	:	706	:	906	:	201	:	105	:	
Maryland																		
(Beltsville)	:	0.2	:	0.1	:	0.2	:	0.1	:	0.1	:	0.1	:	0.1	:	0.1	:	1.0
District of																		
Columbia *	:	5.8	:	8.1	:	1.7	:	2.2	:	2.3	:	3.1	:	2.6	:	0.7	:	26.5
Total	:	6.0	:	8.2	:	1.9	:	2.3	:	2.4	:	3.2	:	2.7	:	0.8	:	27.5

Intramural program is supplemented by extramural support representing (a) 1.2 SMY's at State Agricultural Experiment Stations 1/ and (b) PL 480 funds in two countries representing 218,503 U.S. dollars equivalent.

1/ RPA 210 0.4; RPA 112 0.7; RPA 706 0.1.

\* Sorting and preparation of material to support specialist is also carried on in Missouri, North Carolina, New Jersey, Oklahoma, and Virginia, but does not involve SMYs.

## Problems and Objectives

Only about one-third of the estimated two million or more kinds of insects in the world have been described and named. Many of these are of no known immediate concern to agriculture or mankind, but thousands of species are known to be or are potentially destructive or useful. Minute morphological differences are very important in recognizing many species, and only highly trained specialists are able to positively identify known species and describe new ones. Precise information on the identity and distribution of insects is essential to the efficient conduct of programs concerned with research on harmful insects, the development of methods for their control, and in the management of regulatory activities intended to exclude, control, or eradicate insect pests.

Major objectives of the research are to:

1. Classify and identify insects and mites, including developing new methods and techniques.
2. Detect correlations among taxonomic units on the basis of significant biological characteristics.
3. Determine the fundamentals and characteristics of geographic, host, and seasonal distribution.
4. Develop basic reference works concerning the classification, identification, distribution, and biological characteristics of insects and mites.

## PROGRESS - USDA AND COOPERATIVE PROGRAM

### A. Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites, and Ticks. (9.0 SMY)

1. Moths and butterflies. The scientific name and synonymy of the eggplant leaf miner and other species of the gelechiid genus Keiferia have been clarified as a result of some identifications of these microlepidoptera. Several names have been synonymized with K. inconspicuella, the correct name of this leaf miner. A paper designating more than 25 lectotypes for Nearctic cutworm species in the genus Euxoa has also been completed. (Multiple RPAs)

Two examples of Spodoptera pecten have been identified in light trap material collected at McChord Air Force Base, Washington. The species is common in certain areas of the Orient and is considered a potentially important pest in the United States.

Greatly facilitating a study of the Central American and West Indian cutworm moths is the recent addition to the National Collection of about 3,600 adults from St. Croix, Virgin Islands, and about 2,000 additional moths collected in Panama, Costa Rica, Nicaragua, Honduras, and El Salvador.

By means of a grant to Rutgers University, basic studies on the morphology of insect sense receptors was continued. Experiments were conducted confirming earlier observations that receptors by means of which adults of Trichoplusia ni are induced to lay eggs are present on the antennae. Morphological and histological studies of antennal receptors have disclosed three distinct types, the relative functions of which are being investigated. (RPAs 204, 906)

Basic studies on the taxonomy and biology of immature stages of Acrobasis (family Phycitidae) are being carried out by means of a grant to the University of North Carolina at Raleigh. Fourteen eastern U.S. species have been identified and investigated, and publications regarding this important group will be prepared from the data gathered.

2. Cocroaches, grasshoppers, and allies. The metanotal gland of tree crickets of the genus Oecanthus has been discovered to possess morphological significance in the classification of this insect group. Because the metanotal gland is functional during mating, and because the form and function of the gland are correlated, it is shown to be an especially important indicator of tree cricket behavior.

By means of a PL 480 grant in Uruguay, basic materials have been accumulated to continue already far advanced studies in the taxonomy of South American grasshoppers. This material consists of a collection of 25,000 Neotropical grasshoppers, a complete set of publications, 10,000 file cards containing taxonomic, distributional, and biological data, and over 5,000 photographs and drawings.

The spotted Mediterranean cockroach, Ectobius pallidus (Oliv.), previously known only from Massachusetts in the United States, was found in two areas of Michigan, occasionally invading houses there. Up-to-date information about its bionomics and taxonomic characters was published to aid county agents and pest control operators in its identification. (RPA 706)

Five or more obscure species of cockroaches found asinquilines in Brazilian ant nests have been studied to clarify relationships and limits of higher categories of cockroaches generally and to yield a better understanding of their biosystematics.

Economically important immature grasshoppers are being studied under a cooperative agreement with the University of Idaho. Research is underway to discover taxonomic and biosystematic characters for their separation and identification. Pertinent information has been gathered on about 17 species to date. (RPA 112)

3. Two-winged flies. A new mosquito of the Culex subgenus Melanoconion has been found in Florida crab holes. Also from Florida, an old specimen of Culex latisquama was found in the National Collection; the species had previously not been found north of Honduras.

The rearing of two fruit flies has been significant. One of these, an apparently undescribed species of Chaetorellia, was received from Geneva, New York, having been reared from seeds of Centaurea from Pakistan which had passed through methylbromide fumigation in U.S. quarantine. Chaetorellia species are under biological and taxonomic investigations as promising agents for weed control. The other fruit fly is a species of Ceratitis very closely related to the Med fly, received from Mexico where it had been reared from nutmegs presumably imported from Puerto Rico.

A critical review of the Guttipennis species group of the biting midge genus Culicoides has been published. One of the 10 species of this group, three of which are newly described, is known to bite man; and several have been suspect in animal disease transmission. Another paper describes nine new species of Culicoides from high altitudes in the Colombian Andes. In cooperation with personnel from the University of Florida, Panama Culicoides were also surveyed. Several dozen pints of these adult biting midges were caught in Malaise and light traps in the rain forest, and rearings were made from rarely-found breeding places such as water-holding plants and decaying tropical fruit. (RPAs 210, 706)

The leaf-mining flies of the family Agromyzidae are being studied in a PL 480 grant to India. During the short time the project has been underway, 1,150 adults and 1,500 larvae representing various genera have been collected from a total of 132 host plants, and one paper has been published. The study will make possible the rapid and accurate identification of many of the Oriental groups in this economically important fly family.

4. Parasitic wasps. New hosts, including the mimosa webworm and two species of Agrilus beetles, were recorded for three species of Braconidae; and new State records were noted for 17 species of braconid parasites of lepidopterous and coleopterous larvae and for one pteromalid, Coruna clavata Walker, a secondary parasite of aphids in California.

A chalcid parasite of the introduced bee Megachile rotundata (F.) has been discovered in western Ontario and Utah. The parasite, Pteromalus venustus Walker, is also apparently introduced as it has never before been recorded in North America. (RPA 314)

Continued absence of males from large amounts of material of the mimosa webworm parasite, Elasmus albizziae Burks, has led workers to conclude that the species develops entirely by thelytokous parthenogenesis.

The discovery and description of a new genus and species of encyrtid parasite on the larvae of Forcipomyia constitutes the first record of an encyrtid parasitic on a ceratopogonid midge.

The genus Brachymeria, species of which are parasites of many economically important groups of insects, is being studied by means of a PL 480 grant in India. Although operational for only a short time, this project has already accumulated an impressive amount of literature, specimens, and biological data and will eventually produce means whereby these important parasites can be identified and studied further. In another PL 480 project in India, various genera of the family Ichneumonidae are undergoing taxonomic study. Three new publications arising from this work illustrates, keys, and describes species in three different genera. Significant contributions of Oriental Ichneumonidae have also been made to the U.S. National Collection.

In a third Indian PL 480 project, 28 different genera of Oriental encyrtid parasites were collected and studied. Ten of these genera had not heretofore been collected in India.

5. Beetles. A voluminous amount of data on closely related allopatric beetle populations is accumulating in the course of a joint undertaking with the Bio-Climatic Research Group at Brownsville, Texas. The test insects are the buprestid leaf-miners Pachyschelus purpureus, P. uvaldei, and P. sp.; and the studies are designed to determine the effect of host-plant interchange on biorhythm, developmental rate, fecundity, and food consumption. The results of this study should contribute to a better understanding of genetic versus environmental control of nutritional requirements, host specificity, and diapause.

Continuing studies of the weevil genus Smicronyx along the Caud O Canal indicate the unusual presence of two species on ragweed. Smicronyx usually infests dodder, and in the northern Virginia area being investigated at least seven dodder species are present. These studies are designed to elucidate the possible relationship between host plants and the evolution of the major groups of Smicronyx.

The larvae of the weevil Hypera eximia LeConte were discovered and identified for the first time from collections made from dock (Rumex sp.) at Carrolton, Missouri. Distinguishing characters have been found to enable workers to separate H. eximia from those of all other Nearctic Hypera species.

6. Leafhoppers. An undescribed species of the genus Balbulus has been found in four Maryland localities. A near relative, Dalbulus maidis (DeLong and Wolcott), is the primary vector of corn stunt disease in Texas, California, and Mexico. The new leafhopper species is known to infest gamagrass, Tripsacum dactyloides, a plant known to hybridize with corn; the transmission of corn stunt disease may be affected by the leafhopper in this close association with corn. (RPA 207)

A recently concluded five-year PL 480 grant in Pakistan has resulted in a comprehensive study of Oriental leafhoppers. In all, 63,000 study specimens were amassed along with associated taxonomic and biological data. As a result, over 120 new host plants, some of great economic importance, have been recorded and 107 new genera and 390 new leafhopper species have been or are being described. This study will result in a monograph of the Oriental leafhoppers which for the first time will provide a thorough understanding of this group in the Orient and will clarify leafhopper relationships throughout the world.

About 3,500 leafhopper specimens representing over 100 species were identified in cooperation with an ecological study conducted at the Agricultural Research Center, Beltsville, Maryland. These identifications were of more than usual interest as most of the specimens could be definitely associated with identified food plants, providing new and valuable ecological information concerning many of the species.

7. Whiteflies. Under a PASA arrangement, one of the taxonomic specialists studied the identification and biology of sucking insects on beans and cotton in El Salvador. The aleyrodid Bemisia tabaci (Genn.), an important vector of virus diseases of beans and cotton, is present in small numbers on many weeds surrounding bean and cotton fields in August. Despite these slight initial infestations, the build-up of tremendous populations in November-February is extremely rapid. (RPAs 204, 207)

A newly discovered and described genus and species of whitefly, Venezaleurodes pisoniae Russell, has been discovered to be of considerable economic importance in Venezuela as the transmitter of leaf curl virus of tobacco in that country. (RPA 207)

8. Aphids. Only 14 species of the important aphid genus Macrosiphum had been reported from India until a study of that genus was started in India under a PL 480 grant. Many previously undescribed or unrecorded species have now been found, and taxonomic and biological investigations are underway to provide identification guides and a basis for evaluation of the use of biological control agents against them. (RPAs 112, 204, 207, 906)

9. Thrips. An Argentine thrips has been introduced recently into the United States to help control alligatorweed following intensive biological investigation in its native habitat. It has been described, illustrated, and named as a new genus and species in the family Phlaeothripidae. (RPA 105)

As a result of a PL 480 project in India, 1,478 microscope slides of adult and larval thrips have been added to the National Collection, bringing the total number from that country to about 3,500. Representing many genera and hundreds of Indian species, many of them new, this material greatly enhances the Oriental thrips collection and will be a valuable reference for future identification and research.

10. Mites. A USDA taxonomist has spent two months in Brazil to assist in the development of a graduate training program in acarology at the Agricultural College in Piracicaba. Secondary visits were made to observe and discuss spider mites damaging cotton in the Presidente Prudente area, apple and mushroom mites near Campos de Jordao, and mite-transmitted diseases of citrus in Sao Paulo. Arrangements were also made with a Brazilian acarologist for a cooperative study of Brazilian spider mites. (RPAs 204, 207, 906)

Two new species of erythraeid mites from Canada have been described to aid in a research problem being conducted by the Canada Department of Agriculture in Ontario. A new genus and species of smaridid mite from British Honduras was also described. Many members of both these families are parasitic as larvae and predaceous as adults upon insects and other small arthropods.

11. Aquatic insects. A grant to Oregon State University continues research on the systematics of some aquatic insects that may have value as indicator species useful in determining kinds and degree of pollution present in aquatic environments. Over 300 specimens are now being reared in individual containers to associate larval with adult stages and to accumulate life history data on the various species they represent. Detailed field and laboratory life history studies have been completed on two species of Sialis, between which there appears to be discrete ecological isolation even though the two occur together in the same aquatic habitat.

B. Identification of Insects, Mites, and Ticks. (7.5 SMY)

Authoritative identifications and references to pertinent taxonomic and biological literature are supplied to support Federal and State research, extension, control, and regulatory activities pertaining to entomological problems. These services are also performed for industry, pest control operators, and private individuals in the United States and for foreign agencies and institutions concerned with entomology.

During the year, a total of 29,214 lots of insect material was received for identification. About 348,130 specimens were examined. A total of 111,670 identifications were made and reported. Specimens were accepted for identification only when rendering the service could be justified, as there is a backlog of material awaiting study.

The source of material and the numbers of identifications made of the specimens received from each are shown in the following table:

<u>Source</u>	<u>Number of Identifications</u>	<u>Percent of Total</u>
Agricultural Research Service		
Plant Quarantine Division	28,598	25.61
Plant Pest Control Division	1,288	1.15
Entomology Research Division	8,300	7.43
Forest Service	2,253	2.02
Other Federal Agencies	6,253	5.60
States and Insular Possessions	26,970	24.15
U.S. Individuals	27,305	24.45
Foreign Agencies and Individuals	10,703	9.59
Total Determinations	111,670	100.00

Many of the specimens received for identification are of much interest, either representing new species not previously in the National Collection or documenting new distributional and/or other data. For these reasons, 65,940 specimens of especial value were added to the National Collection during the year.

The systematic review of technical literature essential to the programs in this area included the examination of 2,017 publications which contained 6,486 articles of interest to insect taxonomists. Reference cards (by author) to these articles totaled 9,330. A total of 1,948 was cataloged in depth; and from this effort, 14,089 file cards were made up on which data of significance to taxonomists was recorded. The cards are in continual use in research and service activities, and the file for each specialist is kept up to date and immediately available to him.

During the year, 82 visitors obtained aid on taxonomic, nomenclatural, and other problems. The visitors remained for periods of time varying from an hour or so to several weeks, and came from all parts of the world.

C. Preparation of Keys and Monographs on the Classification, Distribution, Morphology, and Biology of Insects and Related Arthropods. (11.0 SMY)

1. Motns. Taxonomic studies supplemental to a published review of the Nearctic Walshiidae (superfamily Gelechioidea) have been completed. Some species are economically important pests of such diverse plants as peanuts, sweetclover, and ornamental junipers. A manuscript has been prepared with descriptions of 13 new species and a new genus from North America and includes a key for the identification of all the Nearctic species of this family.

Personnel at Cornell University are studying immature stages of cutworm moths (family Noctuidae) under a grant to that institution. At present, most of the taxonomic research centers on the subfamily Hadeninae, of which the larvae of 142 species representing 40 genera have been collected and partially studied.

2. Two-winged flies. The Neotropical species of the family Tephritidae have been cataloged as part of a series entitled "Catalog of the Diptera of America South of the United States." More than 600 species and 100 genera are listed and indexed; citations to the original literature references and sources of current taxonomic information are also given. A total of 12 smaller families of Diptera has also been published in the same series with similar format.

Two tribal revisions in the family Cecidomyiidae have resulted from the discovery of new taxonomic characters. Four generic revisions, two containing keys to and descriptions of aquatic flies, two of parasitic flies, represent significant advances in the knowledge of North American Diptera.

In a cooperative agreement with the University of Maryland, a detailed taxonomic study of the subgenus Neoforcipomyia, genus Forcipomyia (biting midges), including keys, descriptions, and illustrations of the species, has been completed. Observations of an unusual ectoparasitic habit of Forcipomyia larvae attacking Trichoplusia ni indicate that the further studies may prove members of this genus to be of significance as biological control agents. The larvae of most species are terrestrial and feed on fungi.

3. Wasps and sawflies. The American species of the predatory wasp genus Dryudella have been revised. Ten species, two of which are new, are described, keyed, and illustrated; and biological and distributional data are summarized. A similar revision of the parasitic species of Spalangia has also been completed.

Three manuscripts reviewing the sawfly families Blennocampinae, Selandriinae, and Heterarthrinae have been completed. These extensive papers present keys, descriptions, and illustrations of the adults and larvae, many of which are of considerable economic importance due to their leaf-mining habits on forest and fruit trees and other plants.

4. Beetles. A revision of the Neotropical species of the genus Epilachna and a review of the Latin-American epilachnine genus Toxotoma have been nearly completed. Both genera of the plant-feeding coccinellids include important agricultural pest species.

Among the genera of the leaf-feeding family Chrysomelidae, Agasicles and Cryptocephalus have been revised. The former genus contains an introduced Neotropical species which shows great promise in the control of alligator-weed. The genus Cryptocephalus contains 71 Nearctic species and 33 subspecies; the revision presents keys, descriptions, and illustrations to make their identification possible.

A paper providing identifying characters of the important bruchid pest, the bean weevil, and of its relatives in the Obtectus group of the genus Acanthoscelides, has been published. Two other papers describing a new genus and two new species of Neotropical bruchids have also been completed.

Fifty species of seed-weevils have been collected, studied, and included in comprehensive keys to the Bruchidae of the Oriental Region as a result of a PL 480 grant in India. This project has also created a complete taxonomic bibliography on Oriental Bruchidae.

By means of a grant with Northern Arizona University, the biosystematics of the bruchid genera Acanthoscelides and Sennius are being investigated. A manuscript on the western U.S. species of the former genus has been submitted for publication, and representatives of about 50 species of the latter genus are now being studied.

A contract with Texas A&M University has been responsible for a wide-ranging taxonomic and biological study of the boll weevils belonging to the tribe Anthonomini. An intensive taxonomic revision of the pupae in this tribe has

been published with keys, illustrations, and descriptions of 47 species in seven genera. Data from the pupal study will be used to re-evaluate the overall classification of the tribe, heretofore based only on adults; and a separate study on adults will be published in the future. (RPA 207)

5. Leafhoppers. By means of a grant to the University of North Carolina at Raleigh, basic taxonomic studies on female leafhopper classification are being conducted. Important characters of the female genitalia are being correlated with other morphological features, many of them of classic significance for higher category classification, to contribute to the development of a better understanding of systematic relationships among the various taxa of the Cicadellidae.

A complete card index to the published parts of the "Catalog of the Homoptera" has been compiled in a contract with the same institution. This index will be published as soon as the last part of the catalog proper has been published.

6. Scale insects. A taxonomic study of the genus Melanaspis has been undertaken by means of a grant with the University of Maryland. Studies of the adult females and keys for their separation have been completed, and several interesting parasites have been reared from obscure and gloomy scale.

7. Aphids. A list of the North American species of the aphid genus Microsiphoniella has recently been made available. This list, in which there are 13 new name combinations, enables aphid workers to assign correct names to these insects and to place previously incorrectly assigned species in their proper genera.

8. Mites. A book entitled "Spider mites of southwestern United States and a revision of the family Tetranychidae" has been published. It contains a subgeneric revision of this mite family on a world-wide basis, and it presents descriptions of and keys to the spider mites of the southwestern United States, including many new genera and species. This book is the culmination of several years' work and will greatly facilitate the taxonomy of the Acarina.

Illustrated keys to the genera of eriophyoid mites have been developed and similar works for the Tetranychidae and Tenuipalpidae have been initiated. Many species belonging to these groups are of considerable agricultural importance as plant feeders.

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Area No. 16. FOREIGN EXPLORATION, INTRODUCTION, AND EVALUATION  
OF BIOLOGICAL CONTROL AGENTS

USDA and Cooperative Program

Location of Intramural Work:	Scientist Man-Years FY 1968							Total
	Research Problem Area							
	204	207	210	112	201	105		
California	0.5	0.5		3.6		0.4	5.0	
Missouri	2.9	4.7	1.4				9.0	
New Jersey	0.8	2.0	0.4	0.4	0.4		4.0	
Argentina (Buenos Aires)						0.8	0.8	
France (Paris)		2.5	0.5				3.0	
Italy (Rome)				1.8			1.8	
Total	4.2	9.7	2.3	5.8	0.4	1.2	23.6	

Intramural program is supplemented by extramural support representing  
PL 480 funds in two countries representing 220,994 U.S. dollars equivalent.

## Problems and Objectives

Many of the most serious insect and weed pests in the United States have been accidentally introduced from foreign countries without the insect enemies that keep them under control in their native homes. Some of the harmful insects so introduced have been effectively controlled by later introduction of their parasites and predators. Foreign exploration for beneficial biological control agents of insects and their subsequent introduction, colonization, and evaluation in this country is now a well established practice in the control of introduced insect pests. The use of imported insects to control noxious weeds, although a more recent practice, has shown much promise.

Major objectives of the research are to:

1. Discover and introduce natural enemies of harmful insects and weeds.
2. Determine whether such agents will adapt to new ecological requirements or harm beneficial insects or desirable plants.
3. Develop methods for shipping, propagating, and colonizing the agents.
4. Evaluate the effectiveness of the agents in their new environment.
5. Increase by physiological or genetic means the effectiveness of the introduced agents.

## Progress - USDA and Cooperative Program

### BIOLOGICAL CONTROL AGENTS OF INSECT PESTS

#### RPA 204 - CONTROL OF INSECT PESTS OF FRUIT AND VEGETABLE CROPS

#### A. Basic Biology, Physiology, Nutrition, and Evaluation of Biological Control Agents. (3.0 SMY)

##### 1. Vegetables

##### a. Imported cabbageworm, *Pieris rapae*

(1) General. Time required for mass production of *Pieris rapae* has been reduced by utilizing artificial oviposition sites and diets with the aid of attractants. Sinigrin was found to be the best attractant to induce feeding on artificial diets, and crushed mustard leaves in 95% ethanol was an effective attractant inducing oviposition on a strip of plastic material.

Several thousand *Pieris* adults, which had been fed red dye as larvae causing production of pink eggs as adults, were released, and egg counts were made along roadside plots up to 11 miles from the release site. Marked eggs were found as far as 5 miles away from the release sites.

(2) Egg parasites, Trichogramma. It was found that T. evanescens tends to search shaded areas of cabbage plants for host eggs in preference to exposed areas; this species rarely searched non-living surfaces (such as cardboard or wood) for hosts. The native T. minutum appears to find more host eggs in light than dark, and more eggs in oblique rather than direct light. Searching activity of this species increased with increased barometric pressures.

Pieris rapae, an imported pest, appears to be more susceptible to parasitism by the foreign T. evanescens than is the native cabbage looper, Trichoplusia ni.

T. evanescens emerged from some Pieris eggs shortly after collection in October and November, indicating the species may not always enter obligatory diapause in Missouri. Trichogramma did not emerge until Spring from cutworm egg masses from which cutworm larvae emerged in the fall, indicating Trichogramma can diapause in non-diapausing host eggs.

Efficiency of production of Sitotroga cerealella, laboratory host for mass rearing of Trichogramma, has been increased 300% in Missouri. One-inch layers of grain are placed in 2 x 4-ft. hardware cloth cribs and infested with Sitotroga eggs of known age, 1.5 eggs per kernel of grain; 50% of the kernels become infested. The emerging moths are trapped in plastic envelopes.

(3) Larval parasites, Apanteles. Laboratory tests indicate the European A. rubecula is an effective searcher for Pieris larvae. Average parasitization by single females, in numerous replications, of from 1-15 hosts in a 1200 cm<sup>2</sup> cage after 24 hours, ranged from 87 to 96%. Tests indicate the parasite is attracted by both concentrations of host larvae, and by feeding holes. The parasite apparently prefers first instar larvae, but can maintain itself at reduced activity (with increased longevity) on more advanced instars.

An efficient mass rearing technique has been developed for this parasite in Missouri by using artificial diets and containers rather than plant material, resulting in increased numbers of cocoons per female parasite and decreased man-hours of work required.

Adult A. glomeratus from P. brassicae were received in Missouri from Wales and France for study of possible host defense mechanisms on P. rapae.

(4) Pupal parasite, Pteromalus puparum.

(a) Biology. Both unisexual and bisexual types of P. puparum, the only important pupal parasite of Pieris, were found in Missouri; the unisexual type was found to be able to readily change to the breeding bisexual type after 1 or more generations in the laboratory.

Individual P. puparum females produced fewer progeny (from 150 down to 10 progeny produced in life span of the parasite) when the number of hosts (Pieris) provided daily was increased from 1 to 4.

Immature (1-10 day old) P. puparum were successfully stored in host pupae up to 30 days at 2°C (The parasite was also found to successfully overwinter in host pupae in Missouri). Pieris pupae, stored up to 60 days at 2°C, were found to still be acceptable for successful parasitism by Pteromalus. Preliminary data indicate P. puparum finds its hosts more by olfaction than vision.

(b) Release study. Host mortality was 96%, 1 week after the release of 400 adults (200♀♀) of the native P. puparum into a field cage containing 200 large cabbage plants with an average of 8 mature Pieris larvae per plant. Weekly observations over 1 month, showed 100% mortality was obtained - 63% to 80% parasitism, and up to 37% of the host pupae were simply killed by the parasites without subsequent parasitization.

#### (5) Predators.

(a) Biology. A colony of Podisus maculiventris, is being successfully maintained in Missouri. This stink bug is a voracious feeder on lepidopterous eggs and larvae (an average of 3 fifth-instar Pieris larvae were consumed daily by a single pair in the laboratory), and has a high fecundity (a laboratory average of 5 eggs per female per day).

The following predators were noted in a survey of Pieris pupal mortality in Missouri: 2 species of phalangids, 2 species of ants, a pentatomid (Perillus bioculatus), Vespula sp., 2 species of carabids, and a cantharid. Natural predation ranged from 48 to 67% in the field.

(b) Release study. Several thousand adults of the native stink bug predator, Podisus maculiventris, were released in an untreated 1/3 acre cabbage plot in Missouri. Pieris egg and larval mortality due primarily to predation was 56 and 25%, respectively. The corresponding percentages were 72 and 7 in a 1-acre cabbage plot in which parasites and fertile hosts, but no predators were released. This indicates the use of Podisus for mass release may be inefficient.

(6) Pathogens. An as yet unidentified bacterium isolated from cabbage looper larvae, and a microsporidian, tentatively identified as Nosema mesnili, from corn earworm, have also been found to be pathogenic for Pieris rapae larvae.

#### b. Cabbage looper, Trichoplusia ni

(1) Egg parasites, Trichogramma. Preliminary tests in Missouri indicate that eggs of the native cabbage looper are less susceptible to parasitism by the European Trichogramma evanescens than are eggs of the

imported cabbageworm. Other tests indicate that looper eggs 1-11 hours old are the most susceptible age for parasitization by the native Trichogramma minutum. Eggs over 55 hours old were also heavily parasitized or killed even up to eclosion time, but a greater number of these eggs tended to collapse than in the case of young eggs. Looper eggs of intermediate age were not as heavily parasitized by T. minutum (less than 40% as opposed to nearly 100% in the other age groups). Tests showed that although T. minutum stung 24-hour looper eggs, actual oviposition occurred in less than 40%, and apparently some of these were not effectively parasitized. Four strains of T. minutum, maintained on 24-hour old looper eggs, appear to have increased ability to parasitize eggs of this age in succeeding generations (up to 95% in the F<sub>4</sub> generation, with an as yet unexplained drop to 64% in the F<sub>6</sub>). The ability of the parasite to select unparasitized eggs for succeeding attacks was also indicated.

Field dispersal of Trichogramma pretiosum after release was found to be small; looper egg parasitism was 14% at the release point, and only 4% 30 feet from this point.

(2) Pathogens. An as yet unidentified bacterium has been isolated from cabbage looper larvae in Missouri. When administered in artificial diet, it was found to be pathogenic to looper larvae, especially in the prepupal stage. It is also pathogenic for imported cabbageworm. Testing for definitive identification of the bacterium continues.

High populations of cabbage looper were effectively controlled in experimental cabbage field plots in Missouri by a nuclear polyhedrosis virus, at the rate of 60 billion polyhedra per acre applied with standard hand-operated sprayers. Predators and sarcophagid flies were found to be effective mechanical vectors of the virus in the field.

c. Diamondback moth, Plutella xylostella. A laboratory colony of the diamondback moth is being successfully maintained in Missouri on the same artificial diet used for Pieris rapae, and using paper and plastic ovipositional sites. A larval-pupal parasite of the moth (Tetrastichus sp.) is also being studied in the laboratory.

d. Cabbage aphid, Brevicoryne brassicae. A heavy population of cabbage aphid was observed in an untreated quarter-acre cabbage plot in Missouri. The outbreak was controlled within 2 weeks due to heavy attack by coccinellids, syrphids, and the braconid parasite, Diaeretiella rapae. Only 10 cabbage plants proved to be unmarketable.

In laboratory tests, it was found D. rapae prefers medium to large turnip, cabbage, and green peach aphid nymphs. Maximum rate of oviposition by the parasite was obtained when provided with 50 to 60 hosts per day.

2. Deciduous and small fruits and tree nuts.

a. Plum curculio, Conotrachelus nenuphar, and codling moth, Carpocapsa pomonella. Over 76% of the apples in an abandoned orchard in Missouri were damaged by plum curculio, while only 17% were damaged by codling moth. Data indicates a sample of only one quadrant per tree provides an accurate estimate of the population of these two pests, including fruit damage and codling moth larvae removed from under bark.

b. Eastern tent caterpillar, Malacosoma americanum, Tetrastichus malacosomae, Oencyrtus clisiocampae and Telenomus clisiocampae were reared from egg masses of M. americanum in New Jersey. T. malacosomae was the dominant parasite.

3. Citrus and subtropical fruit.

a. Scale insects on citrus. Studies on the ecology and rearing of the natural enemies of 5 important citrus scales continued in Israel under a PL 480 project. Much data was gathered concerning the life history of the little-known Aphytis coheni, a parasite of the California red scale, and biological, ecological, and phenological studies on Tetrastichus ceroplastae, a parasite of the Florida wax scale, were begun. A satisfactory laboratory rearing technique for the latter host was developed. In studies on the interactions of Aphytis hispanicus and Prospaltella inquirenda, parasites of the chaff scale, it was noted that they destroyed more hosts in 1 year when acting separately than they did in 2 years when acting together. Field studies continue on Cheletogenes ornatus, and laboratory studies were begun on Saniosulus ornatus, both acarine predators of citrus armored scales.

b. Coconut rhinoceros beetle, Oryctes rhinoceros. A PL 480 project in India, terminated this year, has gathered information leading to the possible integrated control of Oryctes. Several predators were found to be effective in reducing Oryctes populations, and their biology was studied. Tests indicate that at least one long-lived residual chemical insecticide has little effect on these predators while causing 40 to 60% mortality of the Oryctes adults. These and other studies, including those on attractants and repellents and with the fungus Metarrhizium, indicate that a satisfactory degree of control of the pest can be obtained and maintained through a combination of chemical and biological factors and cultural practices.

4. Cross commodity.

a. Scale insects. Data is accumulating, in research under a PL 480 project in Pakistan, on the relationships between the parasite-predator complex and the host plants of the following scales: Ceroplastes floridensis, Coccus hesperidum, Eulecanium spp., Parlatoria oleae, P. crypta, Pinnaspis strachani, Aonidiella aurantii, A. citrina, A. orientalis, Aspidiotus destructor, Lindingaspis ferrisi, L. floridana, Quadraspidotus armenicus, Q. perniciosus, and Tecaspis sp. Many new hosts for both scales

and parasites have been recorded. Similar studies are planned on the following scales: Antonina graminis, Drosicha mangiferae, Ripersia oryzae, R. sacchari, Saccharicoccus sacchari, Aspidiella sacchari, and others. Biosystematic studies of some of the more important parasites are planned.

B. Receipt, Liberation, and Establishment of Foreign Insect Enemies of Insect Pests. (1.0 SMY)

1. Vegetables

a. Imported cabbageworm, *Pieris rapae*

(1) General. Preliminary data from experiments this spring in Missouri indicate that mass release of both foreign egg and larval parasites in combination with fertile hosts may give effective control of first generation *Pieris rapae*. In a 1-acre cabbage plot, 27,000 fertile butterflies, 1,070,000 *Trichogramma evanescens*, and 6500 *Apanteles rubecula* were released over a period of a complete generation for each species. Estimated egg and larval parasitism of first generation *Pieris* in this plot was 57 and 82%, respectively. Corresponding percentages were 1 and 0 in a plot in which no parasites or hosts were released. Total *Pieris* mortality in the parasite plot, including that due to predation, was 99.2%.

(2) Egg parasites, *Trichogramma*. Small numbers (5,000-25,000) of *T. evanescens* from Europe were released on roadside plots of rape and black mustard in Missouri in the summer of 1967. Egg parasitism was much higher in these plots (20 to 32%) than in check fields (0.02 to 0.07%) throughout the season. (Natural parasitism by native *Trichogramma* in Missouri was also found to be low in spring, 1968, only 0.4%.)

Although field dispersal of *T. evanescens* was found to be small (53% parasitism at release point, 8% at 30 feet and 5% at 100 feet), *Pieris* eggs parasitized by this species were found up to 2 miles from release sites.

(3) Larval parasites, *Apanteles*. The palearctic *A. rubecula* has apparently been successfully introduced into the United States (in Missouri). In the summer of 1967 a stock of this parasite was obtained for release, from its established population in British Columbia, where in many areas it has replaced *Apanteles glomeratus* as dominant parasite of *Pieris*. The parasite was recovered in Missouri in the fall of 1967, in 2 instances as far as 2 to 4 miles from release sites. In spring of 1968 the species was found to have successfully overwintered in a field cage and a release plot (parasitism in the plot was up to 30%). Emergence was better synchronized with host emergence than was that of *Apanteles glomeratus*. Recoveries of F<sub>1</sub> generations of the parasite were also made from new (1968) releases; field parasitism reached 50%. Native hyperparasites, *Gelis* species and *Tetrastichus sinope*, were found attacking both species of *Apanteles* in the field. Small releases of *A. rubecula* were also made in New Jersey and Hawaii in 1967.

b. Vegetable weevil, Listroderes costirostris obliquus. Surveys this year in California indicate that although the Tersilochus species, found for the first time last year, and possibly introduced from South America in 1943-46, was generally distributed throughout southern California, its effectiveness is limited. Average parasitism of the vegetable weevil by this apparently thelytokous, univoltine ichneumonid was only 5%, and rarely exceeded 10%.

## 2. Deciduous and small fruits and tree nuts

a. Comstock mealybug, Pseudococcus comstocki. An infestation of Comstock mealybug was discovered on fruitless mulberry in California in August 1967, the first incidence of this pest west of the Mississippi. Two parasites, Allotropa burrelli and Clausenia purpurea, received from Japan, were released in California in the fall of 1967. Small recoveries of A. burrelli were made the spring of 1968, indicating possible establishment of this species. About 18% of the mealybugs were found to be parasitized by a native encyrtid, Zarhopalus corvinus, in the fall of 1967. About 40,000 Cryptolaemus montrouzieri, a predator obtained from commercial sources, were also released and were very active in the fall, but none of these coccinellids apparently survived the winter.

## 3. Potato

a. Potato aphid, Macrosiphum euphorbiae. Over 300 adult Aphidius avenae bred from the English grain aphid, Macrosiphum avenae in France were sent to Maine for release against its alternate host, the potato aphid.

### RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

#### A. Basic Biology, Physiology, Nutrition, and Evaluation of Biological Control Agents. (3.5 SMY)

##### 1. Forage crops

###### a. Alfalfa weevil, Hypera postica.

(1) General. Some of the major results of the second year of a planned 3-year study in New Jersey and Pennsylvania of the field life cycle of the alfalfa weevil and of the effectiveness of its newly introduced parasites were: development of a procedure for rating the degree of melanization of weevil elytra to allow accurate periodic estimation of the physiological age of weevils in the field; an indication that several weather factors may aid the scheduling of mass collections and the estimation of host populations from field samples; and the accumulation of data on effects of dissimilar climatic conditions and cultural practices on weevil populations, which will aid in evaluating area differences in host populations and parasite effectiveness. The field collection of weevils was aided by use of berlese funnels equipped with a heating element rather than a light source for heat.

The most favorable combination of weevil survival and weevil maturity under storage conditions was obtained by storage at 35°F with a feeding of alfalfa weekly or of 2% sugar solution bi-weekly. A gradual regression in sexual maturity of female weevils (but not of males) had been found to occur in storage. This, and excessive weevil mortality in storage seriously limited production of parasites of the adult weevil. Under the above storage regime, net loss of weevils for parasites, due to both factors, was 30 to 35% after 10 to 16 weeks of storage.

(2) Larval parasites. Difficulty in the laboratory overwintering of both Bathyplectes contracta and B. anurus has severely limited the numbers of these univoltine larval parasites for release in the United States. Data indicate that a somewhat high degree of moisture in the storage environment is essential to ensure viability of the pupae within the cocoons and to secure maximum adult emergence of both species. Data also indicate that, at least in the case of B. anurus, daily temperature fluctuation may be the primary stimulant causing pupation.

Fluctuating daily laboratory temperature has resulted in the production of a greater percentage of non-diapausing cocoons in the case of the bivoltine B. cuculionis. Ordinary laboratory rearing of this parasite, under constant temperature, results in production of 95 to 100% diapausing cocoons. When parasitized hosts were exposed to 77°F for 9 hours and 45°F for 15 hours for the duration of the parasite's developmental time, only 0.5% of the cocoons produced were diapausing.

Research, conducted under a research grant at Cornell University, indicates that larval diapause of Tetrastichus incertus may be in relation to the age of the female parent; progeny of older females tended more to diapause. Diapause terminated in the field in New York about May 20 to 29. About 50% winter mortality of diapausing T. incertus was observed.

(3) Pupal parasites. An attempt this year to breed the eulophid Necremnus breviramulus on Hypera postica prepupae in the laboratory in New Jersey has failed. The parasite had been collected and reared on prepupa of H. rumicis in Missouri. Some initial reproduction on H. postica and a small F<sub>1</sub> generation was obtained in New Jersey, but an attempt to secure further reproduction was unsuccessful. In Europe, Necremnus leucarthros is known to parasitize H. postica pupae.

(4) Adult parasites. The so-called "domestic black Microctonus" was described in a paper published this year, under the name Microctonus colesi. This species, previously confused with M. aethiops, is found in several eastern states and is of unknown origin, being either an adapted native or accidentally introduced foreign parasite. Like the bisexual "European black Microctonus", the females of which are yet to be definitively distinguished from M. colesi, this unisexual species attacks the larva but completes development in the adult weevil, whereas M. aethiops attacks and completes development only in the adult weevil. Studies continue on the

laboratory termination of M. colesi larval diapause; the species was reared from egg to adult in the laboratory for the first time this year. A manuscript has been submitted for publication describing the castration of adult weevils by M. colesi and M. aethiops.

(5) Pathogens. The Moorestown laboratory colony of alfalfa weevils was found to be about 90% infected by a microsporidian, probably a Nosema species. Spores were found in the testes, ovaries, haemocoel, and fat bodies of adult weevils, and in the eggs and larvae. The disease apparently does not kill its host, but drastically reduces egg and sperm production, and has been found to stop pupation of larvae. Studies continue to determine the identity and life cycle of this protozoan.

b. Clover leaf weevil, Hypera punctata. Up to 50% of the clover leaf larvae were parasitized by Biolysia tristis in collections in Arkansas this spring. A single male of this parasite was swept from alfalfa in Pennsylvania in November. B. tristis is almost invariably thelytokous; only 2 males are known to have been previously collected in the United States.

c. Black cutworm, Agrotis ipsilon, dingy cutworm, Feltia subgothica, and bristly cutworm, Lacinipolia renigera. Parasitization of overwintering dingy and bristly cutworms in Missouri was high (over 60%) again this spring. Rogas sp. was reared from the former host and Copidosoma celanae and Apanteles forbesi from the latter, while Microplitis feltiae and the nematode Hexameris arvalis were reared from both hosts. The nematode was also found attacking black and dingy cutworms in Arkansas, the southernmost record of this mermithid to date. A satisfactory method of rearing juveniles of the nematode to adults, with less than 10% mortality, has been devised, involving sterilized and moistened sand-peat moss mixtures with supplemental food.

d. Pea aphid, Acyrtosiphon pisum

(1) General. This was the third successive year that pea aphid populations have been found to be low in alfalfa fields in Maryland, Delaware, and New Jersey. The lack of economic damage by the aphid may be attributed to parasites and disease. Successful overwintering of the aphid, and winter activity of parasites, were found in all areas surveyed except northern New Jersey.

(2) Parasites. Aphidius pulcher was effective this spring, aphid parasitism by this species generally being over 60%. A. smithi, the dominant parasite in the Mid-west and South, was again scarce in the Middle Atlantic region. Hyperparasites, mainly Lygocerus, reduced the effectiveness of Aphidius somewhat. The European pteromalid Coruna clavata was recovered in southern New Jersey in the fall. This is the first record of this hyperparasite in North America south of Maine and New Brunswick, where it has only recently been discovered, attacking primary parasites of potato aphids.

(3) Predators. Predators were common only in Delaware and Maryland, in areas where host populations were sufficient to maintain them. Overwintering populations of the ladybug Coleomegilla maculata were heavily parasitized (42 to 68%) by Perilitus coccinellae in Delaware and New Jersey. Some adults of this ladybug from New Jersey were sent to Ohio State University for study.

(4) Pathogens. A bacterial disease (Bacillus) of the pea aphid was discovered in Maryland this year, apparently the first record of a bacterial disease of Aphidae. The fungus, Entomophthora aphidis, credited with the control of the pea aphid in Delaware and New Jersey in 1967, was absent in the field until June this year, when it infected 4 to 10% of the pea aphids in Maryland and Delaware.

e. Spotted alfalfa aphid, Therioaphis maculata. The first seasonal appearance of the spotted alfalfa aphid in areas surveyed in Maryland, Delaware, and New Jersey, occurred in July in 1967. Indications are that this aphid does not occur north of Trenton, New Jersey. The absence of the aphid during extensive winter surveys in the above 3 states indicates that the species does not overwinter there. All 3 parasites of the aphid, Praon palitans, Trioxys utilis, and Aphelinus semiflavus, were found, as far as known for the first time, in the 3 states in 1967. Parasitism was low (less than 10%), and hyperparasitism extremely low (only 3 hyperparasites were found).

## 2. Wheat

a. Wheat stem sawflies, Cephus spp. A few New Jersey collected Collyria calcitrator were sent to Montana State University this year for laboratory study.

## 3. Corn

a. Attractants for predators. Field tests, conducted under a research grant at the University of Minnesota, indicate that spraying corn plants with sugar solution increases predator populations and reduces European corn borer populations. In another test, using manure as an attractant for predators in corn, larval and adult populations of corn rootworms decreased. Carabid and spider populations apparently were not increased in manured fields, but predatory mite populations showed a sharp increase, suggesting these mites may be responsible for reducing the rootworm populations.

## 4. Cross commodity

a. Cereal leaf beetle, Oulema melanopus. Two native parasites of a native Lema species in the eastern United States were sent to the Plant Pest Control Division laboratory in Michigan for study in regard to their possible application in the control of the cereal leaf beetle. The parasites were the chalcid Spilochalcis delumbis, and the tachinid Paralispe infernalis.

A final report was made upon termination of research on methods of biological control of O. melanopus, under a 3-year cooperative agreement at Purdue University. Voluminous data was collected concerning the life history and biology, and post-release dispersal of the egg parasite Anaphes flavipes, which was liberated in Indiana against O. melanopus during 1965-68. An evaluation of the biological differences of 6 European biotypes of Anaphes (from Nanterre, Tours, and Cahors, France, Lerida, Spain, Rome, Italy, and Zemun, Yugoslavia) was also made. (The population from Tours is presently identified as Anaphes sp. near fuscipennis; all other populations are identified as A. flavipes.) Under laboratory conditions, the biotypes from the 2 southernmost latitudes (Rome and Lerida) tended to develop slowest at 21°C, while those from the 2 northernmost latitudes (Nanterre and Zemun) developed fastest at this temperature. At 15.6°C, parasites from Lerida and Rome developed faster than those from Nanterre and Zemun.

b. Corn earworm-Cotton bollworm, Heliothis zea. Spores of a microsporidian, tentatively identified as Nosema mesnili, were recovered from corn earworm larvae emerging from eggs in Georgia, and from H. zea pupae in California. This is apparently the same Nosema previously recovered from Pieris rapae in Missouri.

c. Carabid parasite-predators. The stimulus, or the larval host, required to elicit oviposition in the laboratory of Lebia analis, has not yet been found. The adults of this ground beetle are important predators in Arkansas of insect pests of cotton, corn, alfalfa, and soybeans. The flea beetle, Disonycha glabrata, found on Amaranthus weeds, is believed to be possibly the larval host of L. analis, but to date no laboratory oviposition of the ground beetle has been obtained. Observation of L. analis under natural conditions continue, under a grant, at the University of Arkansas.

## B. Receipt, Liberation, and Establishment of Foreign Enemies of Insect Pests. (3.0 SMY)

### 1. Forage crops.

#### a. Alfalfa weevil, Hypera postica

(1) Egg predators, Peridesmia discus. Surveys at release sites in Pennsylvania, New Jersey, and Delaware, indicate that P. discus has not yet become established, even after repeated release. Limited funds allowed only small scale collections for this egg predator this year in France; no new shipments or releases were made.

#### (2) Larval parasites.

(a) Bathyplectes spp. The first large scale release of a new alfalfa weevil parasite was made this spring. A total of 3625 adult Bathyplectes contracta were released at 18 sites in Virginia, Maryland,

New Jersey, Pennsylvania, New York, Massachusetts, Vermont, New Hampshire, Ohio, Indiana, and Michigan. The only prior releases of this species, known as "bagged" Bathyplectes due to its peculiar cocoon, were in small numbers in Pennsylvania in 1964 and New Jersey in 1967, with no apparent establishment. B. contracta had been found in small numbers only in the Rhine valley area of France and Germany since 1960. In 1966, the species was found to be more abundant in northern Europe, apparently taking the same place in the weevil parasite complex there that B. anurus does in southern Europe. Mass collection of 200,000 weevil larvae in the summer of 1967 yielded the 32,500 B. contracta cocoons shipped to Moorestown, from which the releases of this year were made. Parasitization of weevil larvae by this species in the Swedish collections reached as high as 59%, as compared with maximums of 41% for B. curculionis, 0.2% for B. anurus, and 9.7% for Tetrastichus incertus.

Surveys indicate a maximum dispersal of B. anurus since its 1963 establishment in Pennsylvania and New Jersey, of only 5 to 6 miles in both areas. Parasitism by this species was greater this year than in the past, especially in Pennsylvania where it exceeded 20% in 12 of 33 samples. B. anurus was dominant in Pennsylvania, whereas B. curculionis was dominant at New Jersey sites except in early season (April) collections. B. anurus was recovered at 3 new release sites in Pennsylvania. Over 12,000 cocoons of the species were collected from Pennsylvania sites to supply release material for 1969. Over 300 B. anurus adults from last year's New Jersey collections were released this year at new sites in Pennsylvania, Virginia, and Illinois. B. anurus was found in Sweden for the first time, but in very small numbers; B. contracta appears to take the same place in the seasonal complex of weevil parasites in northern Europe, that B. anurus does in more southern Europe.

Field parasitism by B. curculionis in the east was disappointing this year, exceeding 20% in only 16 of 59 New Jersey collections and never exceeding 5% in Pennsylvania collections. Additional liberations of this species were made this year: over 1000 adults from established colonies in New Jersey, Pennsylvania, and Massachusetts were released at new sites in Virginia, Massachusetts, Vermont, and Wisconsin, and 13,600 New Jersey collected cocoons of the species were sent to Michigan State University for study and release; over 4200 cocoons, representing the first large collection of B. curculionis in Sweden, provided 1800 adults for release in the northern states of Illinois, Indiana, New York, Massachusetts, New Hampshire, and Vermont, and 118 Swedish cocoons were sent to the University of California for study and release. B. curculionis was found for the first time in Arkansas; its presence there is due to natural dispersal, as no releases have been made in that state.

(b) Tetrastichus incertus. This gregarious parasite has now become established in 11 eastern states of the United States. Data indicate the species may be preventing the development of a large second generation of alfalfa weevil in New Jersey, Pennsylvania, and New York. A total of

5300 host larvae parasitized by this species, collected in these states, were sent to Michigan State University for study and release. Although parasitism by T. incertus was found to be low in Sweden (less than 10%), 367 parasitized hosts and 90 parasite adults were collected, and a laboratory colony was established at Moorestown from which 846 T. incertus adults were sent for release in the northern states of Illinois, Indiana, New York, and Vermont. A survey of the Balkans for alfalfa weevil and cereal leaf beetle parasites, the first such survey undertaken, resulted in the shipment of a few T. incertus from Rumania and Yugoslavia which were subsequently released in New Jersey.

(3) Adult parasites, Microctonus spp. The first large scale release of a new parasite of adult weevils was made this year. Over 1050 adults of the braconid known only as "European black Microctonus" were released at sites in Virginia, Maryland, New Jersey, Pennsylvania, New York, Massachusetts, Vermont, Illinois, and Michigan. The only previous release of this species was in small numbers in Colorado in 1966. Like the "domestic black" Microctonus colesi, this parasite attacks the larva but completes its development in the adult weevil. On the basis of color and structure, males of this species resemble those of M. aethiops, while on the other hand the females cannot be distinguished from those of the unisexual M. colesi. Studies continue leading to differentiation of these closely related Microctonus species. The "European black Microctonus" was obtained from several locations in France, to the east and west of Paris; weevil parasitization by this species reaches 25% in France. Over 300 M. colesi adults were also released in Illinois this year.

Over 3000 New Jersey or laboratory-reared M. aethiops adults were sent for study and release in Mississippi, Tennessee, Kentucky, Maryland, Pennsylvania, New York, Massachusetts, New Hampshire, Vermont, Ohio, Indiana, Michigan, and Wisconsin. Immature stages of this parasite were also sent to the University of Maryland and Pennsylvania State University for study. M. aethiops was recovered from 3 new release areas in Delaware and Pennsylvania and from 2 additional areas in Pennsylvania into which it had spread naturally.

b. "Egyptian alfalfa weevil", Hypera brunneipennis. Surveys this year in parasite release areas in southern California indicate that none of the introduced parasites, Bathyplectes anurus, Tetrastichus incertus, Dibrachoides druso, or Microctonus aethiops, have become established on the Egyptian alfalfa weevil. Only Bathyplectes curculionis was recovered in the surveys. Additional small releases of B. anurus and M. aethiops were made this year, and the first liberation was made of Microctonus colesi, a braconid parasite of adult Hypera. A small number of H. brunneipennis adults parasitized by this species, which was obtained from H. postica in New Jersey, were released in Riverside County.

c. Pea aphid, Acyrtosiphon pisum. This year, 200 Aphidius ervi from France were released against the pea aphid at 2 sites in New Jersey and

Delaware. Some Aphidius from pea aphids in Sweden were also received for study at Moorestown.

C. Search For and Importation of Foreign Enemies of Insect Pests. (3.0 SMY)

1. Forage crops

a. Alfalfa weevil, Hypera postica. A new parasite of the alfalfa weevil adult was discovered this year. A tachinid, Velocia multispina, parasitized up to 10% of the weevils collected in northeastern France in the fall of 1967. In view of the fact that several species of Velocia (=Medina) closely related to multispina have been recorded as parasites of other beetles including beneficial coccinellids and Altica species, this tachinid is currently undergoing host preference tests before consideration can be given to its release in the United States. To date, no oviposition by V. multispina has been obtained in the laboratory on Epilachna varivestis, Ceratomegilla maculata, Hippodamia convergens, or Altica carduorum (the latter is currently being released in North America as a Canada thistle control agent), whereas the species readily reproduces on Hypera postica.

2. Sugar crops

a. Sugarcane borers. A PL 480 project in India is providing valuable data on the role that wild grasses play in the bionomics of sugarcane borers. Several new lepidopterous borers of wild grasses were recorded from India and studies are underway to determine whether these are hosts of parasites recorded from sugarcane borers. A new parasite, Temelucha sp., was found attacking the sugarcane borer, Chilo partellus. Rates of parasitism of the different species of borers were followed throughout the season, and biological observations were made on their insect and nematode parasites, many of which are candidates for introduction into the United States.

3. Corn

a. European corn borer, Ostrinia nubilalis. Two parasites and an unidentified disease of Ostrinia krasmeria, a species closely resembling O. nubilalis but not known to attack corn, have been found as a result of a PL 480 project in India. A technique has been devised to rear O. krasmeria in numbers. This work may result in discovery of some parasites or pathogens useful against the European corn borer in the United States.

4. Cross commodity

a. Cereal leaf beetle, Oulema melanopus

(1) Adult parasites. Over 20% of the adult O. melanopus adults from 2 collections near Chalons-sur-Marne, France, were found to contain a single juvenile nematode. A single adult melanopus from a collection near Soissons contained 8 nematodes. It is not known whether 2 species of nematodes are

involved. No other parasites of adult Oulema have as yet been found.

(2) Egg parasites. Mymarids from Oulema eggs collected near Tours, France, in 1967, and identified as Anaphes sp. near fuscipennis, are now being colonized at Michigan State University, separate from Anaphes flavipes. A shipment of Anaphes from Cologne, Germany, was used in laboratory tests in New Jersey. A spring survey of the Balkans for Oulema parasites, the first such survey undertaken by ARS workers, resulted in the shipment of 85 Oulema eggs parasitized by Anaphes from Austria, Hungary, and Rumania, to the Plant Pest Control Division laboratory at Niles, Michigan, for colonization. An additional 92 parasitized eggs from a new French locality was also sent to Niles. Identification of A. flavipes from field collected material this year indicates the establishment of this parasite in Michigan.

### (3) Larval parasites

(a) General. Over 91,000 Oulema larvae were collected in 1967 (April through August) in Spain, Italy, France, Denmark, and Sweden. From these collections, nearly 12,000 host pupal cells containing diapausing parasites or dead hosts were sent to the United States in addition to over 3500 cells resulting from a PL 480 project in Yugoslavia. Highest field parasitization averages in 1967 were found in the French (51 to 58%) and Danish (34 to 44%) collections; averages in the other collections were 26% in Sweden, 3 to 15% in Spain, and 11% in Italy. (Comparable data for Yugoslavia is unavailable). By the end of June, 1968, over 83,000 additional host larvae had been collected in Spain, Portugal, and France, and over 11,000 Spanish host pupal cells had been sent to Moorestown. All 3 known Oulema larval parasites were found in Yugoslavia, Hungary, and Rumania.

(b) Tetrastichus julis. This eulophid was the dominant parasite in the 1967 collections from Sweden and from 1 area each in France, Denmark, and Spain; average parasitism by the species was 18, 42, 26, and 2%, respectively. Parasitism in the other collections ranged from 1 to 12%. From the host pupal cells collected in 1967 (and the remainder of the 1966 collections), and including some non-diapausing adults from the 1968 collections, nearly 1900 T. julis adults (from all localities except Portugal and Yugoslavia), were sent during the year to Purdue and Michigan State Universities and the Plant Pest Control Division laboratory at Niles, Michigan, for study, propagation, and release. Although T. julis was identified this spring from field collections in Michigan, it is too early to determine whether this species is established there.

(c) Lemophagus curtus. In 1967, this ichneumonid was the dominant parasite in 1 collection in both France and Denmark; average parasitization was 29 and 17%, respectively. In other collections, parasitism ranged from 3 to 16%, except in Spain where this species was not found. Dissections indicate that Mesochorus brevipetiolatus, the hyper-parasite found to date only at Cahors, France, attacks L. curtus. Only 64 non-diapausing adults from the 1967 collections in France and Yugoslavia,

and 3 from this year's survey in Rumania, were sent during the year to Purdue and Michigan State Universities.

(d) "Tersilochus sp.". In 1967, this ichneumonid, tentatively identified as "Temalucha" carinifer, was the dominant parasite only at 2 locations in Spain and in Italy; average parasitization was 11 to 12% and 5%, respectively. In other 1967 collections, parasitism ranged from 0.4% to 16% (in Denmark). This year over 1900 adults of this species, from Yugoslavia, Spain, Italy, and Denmark, were sent to Purdue and Michigan State Universities for study, propagation, and release.

b. Cotton bollworm-Corn earworm, Heliothis zea. Valuable data on parasitism and predation of Heliothis species in India is being collected under a PL 480 program. A nematode, Hexamermis sp., was found to be the most important parasite of Heliothis armigera on tomato; tachinids were next in importance. Shipments of 4 species of parasites (the tachinids "Eucarcelia illota" and Drino imberbis, and the ichneumonids Ecphoropsis perdistinctus and Eriborus sp.) were sent to the United States and forwarded to Arizona for testing and release on the cotton bollworm this year, and the reduviid predator Sycanus indagator was forwarded for testing and breeding on Heliothis to the New Jersey Department of Agriculture laboratory at Trenton.

c. Stink bugs on grains and grasses. A PL 480 project was initiated this year in Pakistan designed to find and evaluate natural enemies of grain- and grass-feeding stink bugs for possible introduction into the United States. In surveys in West Pakistan, 18 species of phytophagous stink bugs were found on various cultivated and wild gramineous plants. (Five of these bugs represent new records for Pakistan). Three species of tachinids (Euthera sp., Plesiocyptera rufipes, and an unidentified species) and a nematode were reared from adults of many of these bugs. Combined parasitism by the tachinids was 2.8% in the foothills surveyed, while only 0.7% in the plains and 0.3% in the hill regions. Parasitism by the nematode was 7.5% in the hills, and only 0.1% in the foothills and 0 in the plains.

d. Grain and forage legume aphids. Preliminary field surveys were conducted in the course of a recently initiated PL 480 project in Pakistan, designed to discover and evaluate natural enemies of legume aphids on grain and forage for possible introduction into the United States. The cowpea aphid, Aphis craccivora, was found to be the most important legume aphid with a wide host range and distribution in zones of moderate climate. The pea aphid, on the other hand, preferred a colder climate and was restricted to fewer legumes. The cotton aphid was a minor pest on legumes as was the yellow clover aphid, although the latter was well distributed. Population sampling techniques were developed and observations on several parasites were made. Continued field observations and the development of techniques for rearing the various aphid enemies are planned.

RPA 210 - CONTROL OF INSECT PESTS OF LIVESTOCK AND POULTRY

A. Basic Biology, Physiology, Nutrition, and Evaluation of Biological Control Agents. (1.6 SMY)

1. Cross Commodity

a. Face fly, Musca autumnalis. High parasitism - over 90% in at least 3 cases - of face fly larvae by the native braconid Aphaereta pallipes was found in late season collections this year in Maryland, Pennsylvania, and New Jersey. Adult emergence of this parasite from field-collected face flies was obtained for the first time in New Jersey. It appears that A. pallipes, a common parasite of dung-inhabiting flies, may be gradually adapting to an introduced host. The parasite may play an important role in reducing overwintering populations of the face fly as evidenced by the high parasitism achieved by this species late this past season.

Heterotylenchus autumnalis, the nematode parasite of the face fly, was found for the first time in 2 additional states this year - Vermont and Pennsylvania. No nematodes were found during the dissection of nearly 300 face fly puparia collected in France this year.

b. Horn fly, Haematobia irritans. Parasitism of horn fly in Missouri from June to November, 1967, averaged only 3.8% (of 8840 puparia collected). Seven species of parasites were reared: Spalangia sp. (? rugosicollis), Muscidifurax raptor, and an unidentified pteromalid (all pteromalid pupal parasites); Neralsia hyalipennis and Xyalophora quinquelineata (both figitid larval-pupal parasites); an unidentified alysiine braconid; and the staphylinid Aleochara sp. (? bipustulata). Spalangia sp., the unidentified pteromalid, and X. quinquelineata were the most common. These are apparently new United States host records for N. hyalipennis, X. quinquelineata, and M. raptor.

For this survey, fastest and most accurate recovery of fly immatures from manure was obtained by either breaking up the patties in a sugar solution, or agitation of the manure in water in a washing machine. In both cases, the immatures were recovered from the surface of the liquids. The use of Berlese funnels for recovery was found to be unsatisfactory.

B. Receipt, Liberation, and Establishment of Foreign Enemies of Insect Pests. (0.5 SMY)

1. Cross commodity

a. Face fly, Musca autumnalis. No evidence of the establishment of Aleochara tristis, the European staphylinid parasite-predator of face flies, was found during surveys this year of release sites in New Jersey, Pennsylvania, and Maryland. Over 2000 adults of the staphylinid, which is thought to be established in Nebraska, were released in Vermont this year.

RPA 201 - CONTROL OF FOREST INSECTS

A. Basic Biology, Physiology, Nutrition, and Evaluation of Biological Control Agents. (0.0 SMY)

1. Mimosa webworm, Homadaula albizziae. The following parasites were reared this year in New Jersey from nests of H. albizziae on honey locust, which was severely damaged throughout the East by this pest in 1967: Elasmus albizziae, Atrometus ?clavipes, Agathis cincta, Eupelmus cyaniceps, Copidosoma sp., Trissolcus sp., Neralsia sp., and Nemorilla pyste. The predominant parasites were E. albizziae and A. ?clavipes. The predator Chrysopa rufilabris was also commonly found in the nests.

B. Search for and Importation of Foreign Enemies of Insect Pests. (0.3 SMY)

1. Smaller European elm bark beetle, Scolytus multistriatus. As a result of collections in Germany and northeastern France, 110 adult Coeloides sp. (probably scolyticida) were sent to the Forest Service laboratory in Ohio and to Michigan State University for rearing on S. multistriatus. The collection from northeastern France yielded the largest number of this braconid, which is not yet established in the United States, that has yet been obtained in Europe. Nearly 2300 Dendrosoter protuberans adults, obtained from Europe, were sent to Ohio and Michigan and 30 Entedon leucogramma also from Europe were sent to Ohio. The former species is now established in Ohio and Michigan as a result of releases made in 1965-66. The latter is already widely distributed in North America probably as the result of an early accidental introduction.

2. Gypsy moth, Porthetria dispar. Six species of parasites were received from Forest Service PL 480 projects in India and Spain and forwarded to the Plant Pest Control Division laboratory in Massachusetts for testing, rearing, and release against the gypsy moth. These included over 1000 Tricholyga segregata adults and over 200 Apanteles sp. from Spain, and 42 Rogas sp., 29 Drino discreta, 56 D. inconspicuoides, and 326 Exorista rossica from India. Small numbers of D. inconspicuoides, E. rossica, and Rogas sp. were also sent to the New Jersey Department of Agriculture laboratory at Trenton for testing.

3. Painted maple aphid, Drepanaphis acerifoliae. Over 150 Missouri collected aphid "mummies" containing the native parasite Trioxys ameraceris, were sent to the University of California at Riverside this year in order to supply this parasite, which is not known to occur in California for release against D. acerifoliae there.

MULTIPLE RPA's

A. Basic Biology, Physiology, Nutrition, and Evaluation of Biological Control Agents. (0.4 SMY)

1. Bagworm, Thyridopteryx ephemeraeformis. The following parasites were recorded this year from overwintering "bags" of the bagworm taken from black

locust in New Jersey: Spilochalcis mariae, Monodontomerus minor, Eupelmus cyaniceps, Eupelmus sp., Habrocytus thyridopterigis, Hymenosyneches thyridopteryx, and Scambus tecumseh. The predominant parasites were S. mariae and M. minor. (201,906)

2. Fall webworm, Hyphantria cunea. Last summer, nearly 1500 adults and immatures of the native H. cunea parasites Apanteles hyphantriae, Meteorus hyphantriae, Campoplex validum, and Mericia ampelus, were sent to Korea University, Seoul, Korea, together with some Itoplectis conquisitor obtained from pupae of Eastern tent caterpillar, for release against fall webworm, recently accidentally introduced into Korea from North America. (201,204,906)

3. Green peach aphid, Myzus persicae. Qualitative and quantitative studies on parasitism of M. persicae are being conducted in northern West Pakistan under a PL 480 project, and observations are being made on alternate hosts of the parasites and on the effects of hyperparasitism. M. persicae has not been found on Prunus in this area of Pakistan, but has been recorded on 29 other hosts. Potato is attacked heavily, but tobacco (Virginia variety) is not a preferred host. (Aphids recorded from Prunus were: Brachycaudus helichrysi, Aphis pruni, and a new species of Myzus). Four species of primary parasites of M. persicae - Aphidius matricariae and Diaeretiella rapae (the 2 most important) and Toxares sp. and an unidentified species - and 5 secondaries - Asaphes vulgaris and a charipine (the 2 most important) and Pachyneuron sp., Tetrastichus sp., and a eulophid - have been recorded. The aphids Hyadaphis erysimi, Myzus dycei, M. ornatus, and Phorodon cannabidis, were recorded as alternate hosts of parasites of M. persicae. (204,207,906)

4. Lygus bugs. Research to determine the role of hymenopterous parasites in regulating populations of Lygus rugulipennis and L. pratensis in Poland continued under a PL 480 project. The only important parasite found has now been identified as Leiophron (or Euphorus) reclinator, not L. pallipes as first reported. L. reclinator, which has not previously been reared from Lygus, emerged mainly from Lygus nymphs in Poland, whereas L. pallipes generally emerges from the adults, in other parts of Europe and the United States. First generation Lygus were more heavily attacked by the parasite in Poland than was the second generation. Undetermined ichneumonids, possibly hyperparasites of Leiophron, were also reared this year. (204,207,906)

5. Tachinid parasites. During field studies concerning the biologies and host relationships of tachinids conducted, under a research grant, at Washington State University, the uncommon Eleodiphaga pollinosa was successfully reared in numbers from the tenebrionid Eleodes hovaverrucula, and a long series of Doryphorophaga doryphorae was reared from the Colorado potato beetle, and about 200 undetermined tachinids were reared from an as yet unidentified moth pest of hops.

About 1500 references have now been indexed leading toward a Parasite-Host Catalog of North American Tachinidae. Additional references are being processed and verified in this important bibliographic endeavor conducted under contract at the California Academy of Sciences, and a manuscript is being prepared. (Multiple RPA's)

6. Aphid predators. Studies under a PL 480 project in India are designed to determine methods of establishing the important palearctic aphid predator, Coccinella septempunctata in the United States. The reasons for the previous unsuccessful attempts have apparently been rapid field dispersal, competition with native coccinellids, and an apparent lack of strong attraction between the sexes of the beetle. The studies indicate dispersal may be curbed by starving the adults to a specific degree before release, and that the age of the beetle may be a factor in dispersal. The preoviposition period may be reduced by mating mature males with newly emerged females. Further study on methods of curbing dispersal, including use of a sugar solution spray, and on competition and host preference are continuing. Although the use of artificial diets alone have proved unsuccessful, the alternate use of artificial and natural diets have aided the laboratory rearing of this beetle. (Multiple RPA's)

7. Development of Superior Parasite and Predator Strains. Colonies of the phytoseiid mite predator, Neoseiulus fallacis, are being maintained at the University of Missouri, with Tetranychus urticae as prey. Factors affecting mortality, fecundity, food consumption, and habitat preference of the predator, and the influence of different prey densities, are being studied under a research grant to determine whether desirable characteristics can be improved and propagated. It appears that this predator can become acclimated to high temperatures (90°F) but that this is not genetically controlled. Alternate food (pollen, etc.) availability is an important factor affecting the reaction of the predator to certain prey densities. (204,207,906)

8. Mass Rearing Techniques. During studies under a PL 480 project in India, which terminated this year, mass rearing techniques were developed for the lepidopterous hosts Heliothis armigera and Prodenia litura, and for their parasites "Carcelia sp." (a tachinid parasite of Heliothis, variously identified as Eucarcelia illota or Carcelia ceylanica) and Chelonus heliopa (a braconid egg-larval parasite of Prodenia). The host rearing was severely complicated by disease which the investigators were able to reduce to manageable levels mainly by rigorous sanitary measures. (One significant observation was the 25% reduction in egg production by Prodenia caused by a microsporidian disease). Important biological and rearing observations were also made on Chelonus formosanus and the ichneumonid Ecphoropsis perdistinctus, parasites of Prodenia and Heliothis, respectively. The 3 hymenopterous parasites were also found to be important enemies of Spodoptera exiqua, the beet armyworm, a pest in the United States. (204, 207,906)

9. Parasite resistance to insecticides. The differences obtained in resistance to metasystox, reported earlier, between control and experimental populations of Trichogramma evanescens and its host Sitotroga cerealella, have been found to lack stability, possibly due to contamination of the laboratory cultures. Steps are being taken to correct for this possibility and tests are continuing. In addition, a new strain of T. evanescens has been isolated from field-collected Pieris, and tests have been started to attempt breeding of resistance to both DDT and metasystox. This work, conducted under a PL 480 project in Poland, is designed to determine the feasibility of developing a strain of parasite that is resistant to toxicants by rearing the parasite on an insecticide-resistant strain of host insect. (204, 207)

10. Forecasting damage by and control procedures for leaf-rollers. A new PL 480 project was initiated late this year in Yugoslavia to study in detail the ecology of 6 species of Tortricidae and 2 of Olethreutidae, all leaf-rolling pests of various crops in Yugoslavia, to determine methods of forecasting crop damage and the necessity, if any, for control measures. (Multiple RPA's)

B. Receipt, Liberation, and Establishment of Foreign Enemies of Insect Pests. (0.2 SMY)

1. Mexican bean beetle, Epilachna varivestis. In summer, 1967, 25,488 adult Pediobius foveolatus were released against E. varivestis in bean and soybean fields in New Jersey, Delaware, Maryland, Virginia, North Carolina, South Carolina, and Alabama. At some sites the parasite became initially established and gave some degree of control. This gregarious larval parasite of Epilachna had been received from India in 1966 at Moorestown, New Jersey, for rearing. A breeding stock of this parasite was sent to the Department of Biological Control, Mexico City, in spring, 1968, for use against E. varivestis in Mexico, where climatic conditions were thought to be more favorable for establishment of the parasite.

In the New Jersey release area, P. foveolatus was attacked by the native hyperparasites Spilochalcis sanguineiventris, Catolaccus aeneoviridis, and Dimmockia incongrua. (204,207)

2. Green peach aphid, Myzus persicae. Two species of parasites were received from India (from a PL 480 project) for testing and release against M. persicae in the United States. Nearly 200 adult Aphidius sp. (possibly matricariae) were released in New Jersey and over 1400 were forwarded to Division laboratories in Washington and Maine. A total of 825 adult Aphelinus gossypii (or "flavipes") was sent to Washington and 687 were released in New Jersey. (204,207)

3. World Review of Introduced Parasites, Predators, and Pathogens. About 1200 manuscript pages of the review of the movement and introduction of biological control agents throughout the world, has now been completed; this

represents about 90% of the proposed review, which is being compiled, under contract, at the University of California at Riverside. (Multiple RPA's)

C. Search for and Importation of Foreign Enemies of Insect Pests. (0.1 SMY)

1. Grasshoppers. As a result of last summer's grasshopper collections in southwestern France, over 700 Acridomyia sacharovi puparia, and 72 Blaesoxipha lineata larvae, were sent to the United States for overwintering. Only 49 adult A. sacharovi have so far emerged for shipment to the grasshopper investigations laboratory at Montana State University. (112,207)

2. Aphids. Much valuable data concerning the biologies and host preferences of 11 species (including 1 new species) of aphidiid parasites found attacking 6 economic species of aphids, and of 4 species of predaceous coccinellids in Taiwan, has been accumulated this year in research under a PL 480 grant. As a result, several of these parasites and predators are being considered for introduction into the United States, especially Aphidius "gifuensis" group and Trioxys communis, which were the most active parasites of the green peach aphid and cotton aphid, respectively, in Taiwan. The information accumulated on the "gifuensis group" may aid in solving a long-standing taxonomic problem with this group of Aphidius.

Similar work under a PL 480 project resulted in the accumulation of considerable data on the rates of parasitism of 6 economic species from many different host plants and localities throughout the season in India. A parasite, Binodoxys aculephae, of the cotton aphid was recorded for the first time from India, and shipments of 2 species of parasites were sent to the United States for study and release on the green peach aphid. A survey of aphidophagous fungi resulted in the first record of Cephalosporium aphidicola for India. (204,207,906)

BIOLOGICAL CONTROL AGENTS OF WEEDS

RPA 112 - RANGE MANAGEMENT

A. Basic Biology, Physiology, Nutrition, and Evaluation of Biological Control Agents. (2.2 SMY)

1. General - Weeds of foreign origin in Idaho. Additional insects were found, and listed, in association with Linaria vulgaris, L. dalmatica, Salsola kali, Halogeton glomeratus, Centaurea maculosa, C. repens, C. solstitialis, and C. diffusa in Idaho, and additional ecological data concerning the Linaria species were gathered under a research contract, at the University of Idaho. The introduced weevil, Cosmobaris americana, appears to be expanding its range in Idaho. The weevil, which feeds on H. glomeratus and S. kali, was found this year on several additional host plants, of the genera Atriplex, Kochia, and Chenopodium.

2. Mediterranean sage, *Salvia aethiops*. Host preference tests in Italy and California with an Italian weevil, *Phrydiuchus* n. sp., indicate that although some adult feeding occurs on several other plants of the Labiatae family (*Ocimum*, *Coleus*, *Mentha*, *Melissa*, *Agastache*, and *Lycopus*), only *Salvia* species, which are the most heavily damaged, are favored for oviposition and larval development. A second new *Phrydiuchus* species, from Turkey, has been found to be much more fecund than the Italian species. The taxonomic descriptions of these 2 species, which are being considered for liberation in the United States against *Salvia aethiops*, are awaiting publication.

3. Musk thistle, *Carduus nutans*. Adults of the Italian flea beetle *Psylliodes chalconera*, being studied as a potential control agent for musk thistle in the United States, have been found to feed on artichoke, safflower and the ornamental sweet sultan in addition to *Carduus* species. However, studies indicate that none of these 3 plants are acceptable as food for the first-instar beetle larvae, nor are sunflower or endive. All evidence so far indicates *P. chalconera* cannot develop on any host plant other than *Carduus*.

4. Tansy ragwort, *Senecio jacobaea*. Studies of host plant damage by, and specificity of the Swiss and Italian flea beetle, *Longitarsus jacobaeae*, have been completed in California. The larvae of this species, which damage plant root crowns, were able to complete development only on 6 species of plants, all annual, biennial, or short-lived perennial plants of the genus *Senecio*. *S. jacobaea* was most heavily damaged. A final evaluation, involving a discussion of conflict of interest, is now being made to determine whether this beetle may be introduced against tansy ragwort in the United States.

B. Receipt, Liberation, and Establishment of Foreign Enemies of Weeds.  
(1.8 SMY)

1. Tansy ragwort, *Senecio jacobaea*.

a. Cinnabar moth, *Tyria jacobaeae*. The established colony of the imported ragwort enemy, the cinnabar moth, at Fort Bragg, California, was drastically reduced this spring. A high moth population was observed in the summer of 1967, and although high egg counts were found in the spring of 1968, the number of larvae was unaccountably low. Possible factors causing this mortality, including a *Nosema* disease and an unidentified ichneumonid pupal parasite, both newly discovered this year, and the tachinid parasite *Lespesia archippivora*, and high mouse and earwig populations, are being investigated.

b. Tansy ragwort seed fly, *Hylemya seneciella*. *H. seneciella* was released in a second California area in the summer of 1967, and for the first time in the state of Washington in the spring of 1968. Adult emergence from the 11,000 puparia collected near Paris last year, was good; shipments

of the fly for release were received at the Southwestern Washington Experiment Station, Vancouver, Washington.

2. Canada thistle, *Cirsium arvense*. The European flea beetle, *Altica carduorum*, established in Alberta, was first released in the United States in 1966-67, throughout the Northwest against Canada thistle. Surveys to date indicate establishment of the beetle in these areas is doubtful. The beetle was released for the first time in the spring of 1968, in Colorado, Minnesota, Wisconsin, Indiana, New Jersey, and Delaware, in an effort to establish the insect in areas receiving more summer rain and higher humidities. Releases were also again made in California, Washington, and Idaho.

3. Toadflax, *Linaria* species. Last summer a small number of pupae of the moth *Calophasia lunula*, was sent for release in Idaho for the first time, against the introduced weed, *L. vulgaris*. The species had been bred on *L. dalmatica* in the California laboratory from material received from Canada in 1966.

C. Search for and Importation of Foreign Enemies of Weeds. (1.8 SMY)

1. Russian thistle, *Salsola kali*. An unidentified moth larva was found in Italy feeding upon *S. kali*, as a miner in early stages, living in stems feeding on buds. Later instars are capable of almost completely destroying the seeds. This insect appears to deserve further study as a candidate for introduction into the United States.

2. Dyer's woad, *Isatis tinctoria*. Several possible candidates for introduction into the United States against Dyer's woad, were found in Italy this year, including 2 curculionids, a chrysomelid, and a buprestid.

3. Hoary cress, *Cardaria draba*. During a 1967 survey of the Middle East for enemies of cruciferous weeds, especially *C. draba*, 2 beetles, a curculionid and a chrysomelid were found to show promise as control agents.

4. Witchweed and other weeds. A PL 480 project was initiated to find and evaluate natural enemies of witchweed and nutsedge, as well as of several aquatic weeds, in India, for possible introduction into the United States. This new project is a continuation of a similar PL 480 project terminated early in 1968.

5. Weeds common to the United States and Pakistan. The identification of many weeds and their enemies, and ecological, distributional, biological, and host specificity studies conducted under a PL 480 project in Pakistan, has made it possible to eliminate many insects as possibilities for introduction into the United States as weed control agents. Three lepidoptera, tested on Halogeton, have not proven promising; 2 curculionids, *Baris scolopacea* and *Neobaris* sp., received from the USSR, are currently being screened on this weed in Pakistan. Two lepidoptera, a beetle, and a hemipteron, found on Xanthium, are now believed to be of doubtful value. *Melanagromyza cuscutae*

(agromyzid) and Smicronyx cuscute (curculionid) (both species already sent to Barbados for field testing), and Eupoecilia ambiguella (phaloniid), Psorosa strictella (pyralid), Eublemma sp. (noctuid), and Trypanea stellata (tephritid) remain promising as enemies of Cuscuta, while tests have eliminated 2 other species of lepidoptera. Tests also continue on Thressa sp. (chloropid), Bactra spp. (tortricids), and Athesapeuta cyperi (curculionid) on Cyperus, and Terellia serratulae, Tephritis sp., and Urophora sp. (all tephritids), and Homoeosoma binaevella (pyralid) on Carduus.

6. Thistles. The most important natural enemies of thistles found this year in surveys in Egypt, under a PL 480 project, were: an unidentified moth ("plutellid?") which severely damaged Salsola kali; 3 species of tephritids (Acanthiophilus helianthi, Chaetorellia succinea, and Urophora macrura) infesting Centaurea calcitrapa; and 2 cerambycids (Agapanthia dahli and A. lateralis) on Carduus pycnocephalus (and also Centaurea calcitrapa in the laboratory). Biological and host preference studies of these insects are underway. All represent possible candidates for introduction into the United States.

#### RPA 105 - CONSERVATION AND EFFICIENT USE OF WATER FOR AGRICULTURE

##### A. Basic Biology, Physiology, Nutrition, and Evaluation of Biological Control Agents. (0.4 SMY)

###### 1. Alligatorweed, Alternanthera phylloxeroides

a. Alligatorweed flea beetle, Agasicles. Results of experiments in which a phosphorus deficiency was induced in laboratory cultivated alligatorweed showed that the absence of phosphorus significantly reduced the feeding fecundity of the flea beetle.

b. Alligatorweed thrips. A manuscript has been prepared providing a name and taxonomic description for this recently released alligatorweed enemy. In the laboratory, the larval stage of this thrips was determined to cover a period of 28 to 29 days.

2. Waterhyacinth, Eichornia crassipes. Studies of the life history of the noctuid, Arzama densa, found infesting 20% of the E. crassipes plants in Louisiana, have been completed, and a weevil, Sphenophorus sp., has been found feeding on this aquatic weed. Studies of these 2 species as they relate to the control of waterhyacinth, continue under a research grant at Louisiana State University.

##### B. Receipt, Liberation, and Establishment of Foreign Enemies of Weeds. (0.4 SMY)

###### 1. Alligatorweed, Alternanthera phylloxeroides

a. Alligatorweed flea beetle, Agasicles. The flea beetle is effectively

controlling alligatorweed in the Jacksonville, Florida, release areas, and continues to disperse. As a consequence, the U.S. Corps of Engineers has halted all alligatorweed spray operations in Florida for 1968, in order to allow the beetle population to increase and spread. However, in the Savannah, Georgia, release area, the beetle population remains low and rather ineffective, although some dispersal of the beetle is taking place. Agasicles was found to be established and showing promise at other release sites surveyed this year, as follows: 4 of 5 sites in South Carolina, 1 of 3 in Alabama, 1 of 3 in Mississippi, 1 of 2 in Texas, 1 of 4 additional sites in Florida, and at all 3 additional sites surveyed in Georgia. Agasicles grubs were attacked by an unidentified dipterous parasite in South Carolina and Georgia, and both grubs and adults were preyed upon by the stink bug, Podisus mucronatus, in Florida. New releases of Agasicles (over 500 adults) were made this year in California and Texas.

b. Alligatorweed thrips. This year, over 6200 alligatorweed thrips were released at several sites in Florida and Georgia, and 6000 were released in South Carolina, 2500 in Mississippi, and 500 in California, at single release sites. In later surveys, the thrips was found at 2 of the new release areas in Florida and 1 in Georgia. Observations confirmed the establishment, and some dispersal, of the thrips at the previous release site near Jacksonville, Florida.

C. Search for and Importation of Foreign Enemies of Weeds. (0.4 SMY)

1. Alligatorweed, Alternanthera phylloxeroides. The phycitid moth Vogtia malloi, the last of the 3 major alligatorweed enemies found in South America, was cleared for introduction under quarantine into the United States this year. Shipments of V. malloi from Argentina are being made to California, where the moth will undergo final host preference and feeding tests leading to its possible eventual liberation in the United States against alligatorweed.

2. Waterhyacinth, Eichornia crassipes. Field observations in Argentina found waterhyacinth to be attacked by a species of grasshopper, an oribatid mite (Orthogalumna terebrantis), a weevil (Neochaetina bruchii), and a pyralid moth (Chilo ignitalis). (Identifications are preliminary). Biological studies and host specificity testing of the mite and the weevil have commenced. Only preliminary observation of the moth, which was found only during cool weather collections, has been conducted. All of these species are candidates for introduction into the United States as biological control agents of waterhyacinth.

3. Water primrose, Jussiaea repens. Field surveys in Argentina found water primrose to be attacked by 2 species of flea beetles, a leaf beetle, an oedemerid beetle, a weevil, and a thrips, all species as yet unidentified. Preliminary biological studies and testing of the thrips and the weevil have commenced. These 2 species, and the leaf beetle, which was found only during warm weather collections, show promise as possible control agents of water primrose for introduction into the United States.

4. Eurasian watermilfoil, Myriophyllum spicatum. Four species of insects have been found to be promising candidates for introduction into the United States as biological control agents of this aquatic weed, as a result of a PL 480 project in Pakistan. These 4 species, Bagous geniculatus, B. vicinus, and Phytobius sp. (all curculionids) and Aristotelia sp. (a gelechiid) are currently undergoing host specificity and other biological tests in Pakistan.

In a similar PL 480 project in Yugoslavia, 4 different promising natural enemies of watermilfoil have been found: Cataclysta lemnata, Parapoynx stratiotata, and Nausinoe nymphaeata (all pyraustids) and Donacia sp. (chrysomelid). In the case of the lepidoptera, preliminary observations on their life histories and relationships to watermilfoil have been conducted. The distribution of Myriophyllum has been determined as a result of the survey in Yugoslavia.

Publications - USDA and Cooperative Program

BIOLOGICAL CONTROL AGENTS OF INSECT PESTS

RPA 204 - CONTROL OF INSECT PESTS OF FRUIT AND VEGETABLE CROPS

Basic Biology, Physiology, Nutrition, and Evaluation of Biological Control Agents

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- Parker, F. D., and Bohart, R. M. 1968. Host-parasite associations in some twig-nesting Hymenoptera from Western North America. Part II. Pan. Pac. Entomol. 44(1): 1-6.
- Rosen, David 1967. The hymenopterous parasites of soft scales on citrus. Beitr. z. Ent. 17(1/2): 251-279, illus.

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

Basic Biology, Physiology, Nutrition, and Evaluation of Biological Control Agents

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- Puttler, Benjamin 1967. The interrelationship of Hypera postica (Coleoptera: Curculionidae) and Bathyplectes curculionis (Hymenoptera: Ichneumonidae) in the Eastern United States with particular reference to encapsulation of the parasite by the weevil larvae. Ann. Ent. Soc. Amer. 60(5): 1031-1038, illus.
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Search For and Importation of Foreign Enemies of Insect Pests

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- Jai Rao, K, and Baliga, Hemlata. 1968. Sturmiopsis inferens Towns., a tachinid parasite of sugarcane and paddy stem borers. CIBC Tech. Bull. 10: 33-48, illus.
- Manjunath, T. M. 1968. New records of some parasites and predators of the rice mealy-bug, Ripersia oryzae Green (Hemiptera: Pseudococcidae). Curr. Sci. 37(12): 354-356.
- Mathur, K. C. 1967. Notes on Banchopsis ruficornis Cameron (Hymenoptera: Ichneumonidae) an internal larval parasite of Heliothis armigera (Hübner) in India. Curr. Sci. 36(13): 356-357.
- Patel, R. C., Singh R., and Patel, P. B. 1968. Nuclear polyhedrosis of the gram pod borer, Heliothis armigera. Jour. Econ. Ent. 61(1): 191-193.
- Saxena, A. P. 1967. Biology of Campyloneurus mutator (Fabricius) (Hymenoptera: Braconidae). CIBC Tech. Bull. 9: 61-72, illus.
- Subba Rao, B. R., Parshad, B., Ram, A., Singh, R. P., and Srivastava, M. L. 1967. Further notes on Hypera postica (Gyllenhal) and its natural enemies. Indian Jour. Ent. 29(4): 370-379, illus.
- Subba Rao, B. R., Sharma, A. K., and Saxena, J. D. 1967. The identity of Apanteles sp. parasitizing Chilo zonellus in India. Bull. Ent. 8(1): 64-66, illus.

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#### Search For and Importation of Foreign Enemies of Insect Pests

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#### BIOLOGICAL CONTROL AGENTS OF WEEDS

##### RPA 112 - RANGE MANAGEMENT

#### Receipt, Liberation, and Establishment of Foreign Enemies of Weeds

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- Baloch, G. M., Mohyuddin, A. I., and Ghani, M. A. 1967. Biological control of Cuscuta spp. II. Biology and host-plant range of Melanagromyza cuscudae Hering (Diptera: Agromyzidae). Entomophaga. 12(5): 481-489, illus.
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- Sankaran, T., Srinath, D., and Krishna, K. 1967. Haltica caerulea Oliver (Coleoptera: Halticidae) as a possible agent of biological control of Jussiaea repens L. CIBC Tech. Bull. 8: 117-137.

AREA NO. 17 INSECT PATHOLOGY

USDA and Cooperative Program

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Location of	Scientist Man-Years FY 1968					
	Research Problem Area					
Intramural Work	112	: 20 <sup>4</sup>	: 207	: 210	: 906	: Total
Maryland (Beltsville)	1.0	: 3.0	: 4.0	: 1.0	: 1.0	: 10.0

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Intramural program is supplemented by extramural support representing

1.0 SMY at State Agricultural Experiment Station.<sup>1/</sup>

<sup>1/</sup> RPA 207 - 0.7, RPA 314 - 0.3

## Problems and Objectives

Control of injurious insects by use of chemical insecticides is an increasingly difficult problem due to the development of resistance in insects to certain insecticides and the need to avoid harmful residues in and on food and forage crops and in the environment. Broad spectrum insecticides are frequently destructive to insect parasites and predators and other beneficial species. Many insect pathogens occur in nature but, except for occasional epidemics, do not exert sufficient control to provide adequate protection to permit full production of marketable quality farm products.

Objectives of the research are to determine:

1. Efficiency of selected insect pathogens, including viruses, protozoa, fungi, bacteria and bacteria-carrying nematodes.
2. Mode of action and routes of transmission.
3. Mammalian safety.
4. Methods for mass production.
5. Optimum conditions for storage and use.

## Progress - USDA and Cooperative Programs

### Multiple RPAs

#### A. Viral Disease of Insects (4.4 SMY)

1. Serological studies. Antisera have been prepared for the following viral materials: Spodoptera frugiperda polyhedral proteins and virions; Estigmene acrea polyhedral proteins and virions; Trichoplusia ni polyhedral proteins and virions, both the single embedded virus (SEV) and multiple embedded virus (MEV) varieties; and Bombyx mori nuclear polyhedrosis virions.

Antibodies against viral products have been demonstrated best by the Ouchterlony immunodiffusion and the complement-fixation (CF) tests. Serologic reactions have not been observed by ring test (simple capillary diffusion) or by the Oudin test (agar capillary diffusion). Immunoelectrophoresis methods are being standardized.

Precipitin reactions in Ouchterlony diffusion tests, using soluble antigens, have been demonstrated by indirect fluorescent antibody technique. Preliminary experiments suggest that this technique may be adaptable to the demonstration of insoluble, immobile antigens, e. g. virions.

There are indications that Bombyx and possibly other tissue culture antigens have complement binding activity under some condition. A study of the complement fixing activity of normal insect hemolymph is nearing completion, subject to the availability of insects.

Experiments, following injection schedules published for producing antibodies against mammalian tissue cultures, have not demonstrated maximum antibody production against insect cell lines. The method of preparation of antigens from insect tissue cultures is being modified.

In progress are studies of the serological relationships between the polyhedra of T. ni SEV and MEV varieties and those of H. zea, a SEV variety. The results of experiments utilizing absorbed and cross-absorbed antisera with the fluorescent antibody technique are encouraging. Similar experiments employing immunodiffusion and immunoelectrophoresis procedures are being performed concurrently.

2. Histopathological studies. Histopathological investigations (using light and electron microscopy) were conducted on several insect species with previously undescribed viruses (polyhedral and granulosis) as summarized below:

	<u>Zebra caterpillar,</u> <u>Ceramica picta</u> (Harris)	<u>Almond moth,</u> <u>Cadra cautella</u> (Walker)	<u>Indian-meal moth,</u> <u>Plodia interpunctella</u> (Hubner)
Type of virus	Nuclear polyhedrosis	Nuclear polyhedrosis	Granulosis
Size of virus	1.5 - 22 $\mu$	0.95 - 1.8 $\mu$	252 x 431
Size of virions	Rod-shaped 40 x 312m $\mu$	Rod-shaped up to 32 x 300m $\mu$	Rod-shaped 25m $\mu$ x 224m $\mu$
No. virus rods within a membrane	1 to 13	2 to 24	1
Susceptible tissues	Tracheal matrix hypodermis fat body	Tracheal matrix hypodermis fat body Malpighian tubules anterior midgut tracheal and sheath tissues of reproductive organs and muscle	Tracheal matrix hypodermis fat body
Virulence	Highly susceptible	-----	-----

Ceramica picta: Many attempts made to obtain dosage mortality data on the zebra caterpillar were complicated by problems of contaminated rearing stock and lack of a suitable semi-defined diet. This nuclear polyhedrosis virus is very virulent. Controls always had a relatively high mortality although every effort was made to eliminate contamination. The virus is apparently transmitted transovarially. Light microscopic studies on late instar larvae have revealed that sheath tissues and tracheal matrix cells of the reproductive organs were affected. Cross transmission studies of non-host species inoculated with nuclear polyhedral suspensions from zebra caterpillar have given negative results with the following species in laboratory tests: fall armyworm, Spodoptera frugiperda (J. E. Smith); greater wax moth, Galleria mellonella (L.); southern armyworm, Prodenia eridania (Cramer). In 5 tests with corn earworm, Heliothis zea (Boddie) and tobacco budworm, Heliothis virescens (Fabricius), we obtained 5 to 10% mortality and delayed preparation (2 weeks). Light microscopic examinations of tissues from these larvae revealed a heavy infection of cytoplasmic polyhedra and light to moderate infection in hypodermis, reproductive organ sheath tissues, fat body, and tracheal matrix. T. ni larvae (laboratory reared on semi-determined diets) demonstrated very high susceptibility to this virus. In cooperation with the Yakima, Wash., laboratory, a small field study was conducted in which larvae of variegated cutworms, Peridroma saucia, were inoculated with nuclear polyhedra of zebra caterpillar. Sections of larvae obtained on successive days thereafter showed polyhedral virus in 6 days with heavy infections by 9 days in nuclei of the hypodermis, tracheal matrix, and fat body tissues.

Cadra cautella: In the almond moth studies, along with polyhedral formation, a great increase in the production of protein by the infected cells which was not all utilized in the process of polyhedral formation was noted. This was not observed in the tissues of control larvae.

Plodia interpunctella: In studies on the Indian-meal moth in late stages of infection some abnormal granulosus capsules were found, although the majority of the virus produced showed a typical morphology. The abnormal capsules were considerably larger in diameter due to greater amounts of protein encapsulating the virions; some had 2 or 3 virions per capsule; other capsules were 2 or 3 times the normal length but of normal diameter.

3. Cultivation of insect viruses in aviary hosts: Attempts to cultivate insect viruses in bird eggs proved unsuccessful. Both nuclear and cytoplasmic viral inclusion bodies and free viral particles were used as the inoculum.

4. General: Slides for light microscopic examination have been prepared for study of pathogenesis of polyhedral viruses in the corn earworm, H. zea, beet armyworm, Spodoptera exigua, and cabbage looper, T. ni. Many other slides were prepared for other workers in the laboratory.

Preparation of virus rods and polyhedral protein for serological and animal tests were made from samples of viruses obtained from the following: Spodoptera frugiperda, Spodoptera exigua, H. zea, T. ni, and Prodenia ornithogalli Guenée. Large samples of viruses produced under contracts have been isolated from larval debris, purified and freeze dried.

In the course of investigations on virus infected tissues we have observed the presence of the contaminant in many species which we suspect to be a microsporidian. Studies are in progress to obtain ultrastructural details on its morphology at different stages of development.

Considerable time has been devoted to examinations of pellets of polyhedra obtained from T. ni from different sources and to laboratory studies of passages through non-host species.

## B. Protozoan Studies

1. Nosema apis. Field tests were conducted to determine the efficiency of heat treating nosema-contaminated bee equipment as a means of eliminating this disease from the colony. The tests were also designed to show the difference in production between infected and non-infected colonies.

All frames used in the tests were sprayed with viable nosema spores. Half of these were heated at 120°F for 24 hours.

Infection was observed in the bees on the non-heated equipment within two weeks; infection peaking at the 5th week. No infection was discovered in the bees on heat-treated equipment. At the end of 14 weeks the non-infected colonies had a weight increase of 45% over those colonies infected with nosema.

These and similar tests run during the past three years demonstrate conclusively that nosema can be eliminated from the bee colony by this single inexpensive method of heat-treatment and also that nosema-free bees out produce infected bees. If nosema disease were eliminated, a conservative estimate of the increased honey production on the national level would be 18 million pounds per year and valued at 2.2 million dollars.

## C. Insect Tissue Culture

1. The use of enzymes in the preparation of insect tissue for primary culture. The treatment of ovarian tissues from the fall armyworm, Spodoptera frugiperda, with the enzymes, trypsin and hyaluronidase, substantially improved the production of primary cell cultures. The trypsin treatment produces free cells and small aggregates of tissue from which additional free cells readily migrate. The extensive damage done to insect cells by the more rigorous treatments normally used for vertebrate

cells was greatly reduced by using a low concentration of trypsin and by incubating at room temperature for only a short time. However, as a result of this treatment the tissue is very sticky and difficult to transfer. Treatment of the tissue with hyaluronidase eliminated the stickiness and further dissociated the cells. The combined treatment provides some free cells and many partially dissociated tissue fragments.

2. Development of a convenient microculture method for insect tissue culture. A method has been devised for efficiently producing primary culture of insect tissues. Finely minced ovarian tissue was cultured in the 96 individual wells of a plastic, disposable, microtitration plate. Five or six insects will provide a complete set of 96 cultures compared to 24 insects for similar number of tube cultures. Four or five insects are required for a single flask culture.

Preliminary studies have shown that the cultures support normal replication of some of the insect viruses and attempts are being made to develop standard procedures for virus assay using these cultures.

#### D. Bacterial Pathogens of Insects (5.6 SMY)

1. B. thuringiensis B. T.  $\delta$  endotoxin. A method for standardizing Bacillus thuringiensis  $\delta$  endotoxin preparations which eliminates the use of the insect has been developed. The procedure makes use of the ability of solubilized  $\delta$  endotoxin to inhibit the enzyme trypsin. When the inhibitory dose (In50) is plotted on ordinary graph paper as the ordinate axis and the lethal dose (LD 50 at 72 hours) obtained after oral injections of the  $\delta$  endotoxin into larval silkworm Bombyx mori as the axis of abscissas, a straight line relationship occurs which can be used in predicting the LD 50 for silkworm after determining the In50 of a preparation. Since a number of other lepidopterous insects react differently to any particular variety of Bacillus thuringiensis  $\delta$  endotoxin it has been suggested that trypsin inhibition alone be used as a basis of comparison of the varieties of  $\delta$  endotoxin produced.

The effects of solubilized  $\delta$  endotoxin produced by Bacillus thuringiensis var. dendrolimus on the hemolymph of the silkworm, Bombyx mori, was studied. When hemolymph was mixed with two fold serial dilutions of the toxin at the pH of the hemolymph, a complex and precipitant occurred and was dependent on the concentration of  $\delta$  endotoxin to hemolymph protein. As the concentration increased further, the amount of total precipitate gradually leveled off. Injection into the hemocoel of larval silkworm produced an LD 50 of 0.0067  $\mu$ g/mg insect at 48 hr. Undissolved parasporal crystals had no effects on the hemolymph.

#### E. Disease Diagnosis

1. Fungus Infections. Of the total number of accessions received for disease diagnosis during the year, 47 percent contained insects on which saprophytes or entomogenous fungi, or both, were found. Entomogenous

forms included a number of species of Entomophthora: E. muscae Cohn from 2 accessions of Musca domestica; E. coronata (Cost.) Kevorkian from Culex quinquefasciatus; E. Grylli Fresenius from Prosapia bicincta; and E. fresenii Nowakowski (Thaxter) from a species of Tetranychus mite. Spicaria rileyi (Farlow) was found infecting Trichoplusia ni, Pseudoplusia includens, and Plathypena scabra. Another (unidentified) species of Spicaria was found infecting Spodoptera frugiperda. In addition a species of Myriangiiales was found in Pseudoplusia includens.

Fungi ordinarily considered saprophytic were found on a number of insects from accessions received; these included: Aspergillus flavus from Diatraea saccharalis (Fab.), Drosophila melanogaster Meigen, Platynota sultana Walsh, and Laphygma exigua Hb.; Aspergillus niger and a Penicillium sp. from Tetranychus mites; and unidentified species of Cladosporium, Rhizopus, Spermospora, Mucor, and Fusarium from dead insects from several accessions.

In addition an unidentified yeast was isolated from laboratory-reared Diatraea saccharalis submitted for disease diagnosis by the Puerto Rico Nuclear Center, Mayaguez, Puerto Rico. Tests conducted with this isolate indicated that it is a low-grade pathogen with minimal invasive powers under normal conditions. However, these studies also suggested that if this organism can gain entry, in sufficient numbers, into the hemocoel, it will reproduce, grow, and cause insect mortality. When mature wax moth larvae were injected with  $9 \times 10^7$  yeast cells (per insect) 60 to 70 percent mortality occurred. No mortality occurred in control larvae injected with sterile distilled water. Examination of moribund larvae showed silk glands peppered with dark lesions, and encapsulated clumps of phagocytized yeast cells were observed in the region of the thorax. The fat tissue also showed spotty, darkened areas. Histological preparations from some of the original Diatraea specimens submitted and from wax moth larvae injected with the organism, revealed a similar pathology. All but the fungicidal agent Nystatin proved ineffectual against the yeast, but Nystatin proved readily inhibitory to both the original isolate and to the same after passage through wax moth. Further studies will be carried on to determine whether the yeast is solely responsible for the mortalities occurring in laboratory colonies. Such studies would seem warranted considering the report that similarly-infected larvae were observed among field-collected specimens in Puerto Rico.

Isolation of a number of the above fungi were obtained and added to our culture collection along with permanent slides.

2. Protozoan infections. Accessions containing insects infected with protozoa comprised 14 percent of those received for disease diagnosis. Two accessions received from the Institute of Agriculture, Anand, India, contained insects infected with microsporidia. One accession contained 7 dead, laboratory-bred sunn hemp caterpillars. Utethesia pulchella, from which a purified suspension containing a total of  $2.4 \times 10^9$  spores was

obtained. The greater part of this suspension was fed to Heliothis zea larvae but no infection occurred. The remainder of the suspension was used against mature larvae of Hyphantria cunea. The latter were injected with spores treated at various times (0, 2, 4, 8, 16, 32, and 77 minutes) with 0.02 M and 0.036 M NaOH. No noticeable symptoms were observed during the course of the test, but on the 17th day, at which time all surviving insects were pupae, all specimens examined (at all exposure times) showed a light to moderate microsporidian infection.

The other accession submitted from the Institute contained dead, laboratory-bred larvae of Laphygma exigua heavily infected with a microsporidian, probably Nosema laphygma. Both feeding and injection tests were conducted using Spodoptera frugiperda as a test animal. No infection was obtained by feeding, and only one pupa from the injected series showed spores in any numbers exceeding the  $3.07 \times 10^5$  spores (exposed to 0.04 M NaOH for 4 minutes) originally injected.

In addition, an unidentified species of microsporidia was found infecting laboratory-reared larvae of Platynota sultana Walsh submitted by the Vegetable and Ornamental Plant Insects Investigations at Beltsville.

A large shipment of laboratory-reared Heliothis zea larvae, removed from a colony started from eggs obtained from a new source, was submitted by the Southern Grain Insects Research Laboratory, Tifton. Although these insects exhibited symptoms suggesting a virus or microsporidian infection, careful examination of over one half of the specimens revealed no disease.

In an accession received from the Ministry of Agriculture, Jaffa, Israel, oocysts of a coccidian identified as a species of Adelina were observed in living and dead larvae of Trogoderma inclusum.

Another interesting protozoan was observed in a scarabaeid, Phyllophaga anxia, submitted by the University of Nebraska at Lincoln. Examination of the fixed material revealed the presence of a number of sporodins of an unidentified cephaline gregarine.

3. Bacterial Infections. Although over 100 bacterial isolates were obtained from insects received for disease diagnosis during the year, only 14 percent of those tested proved to be virulent pathogens. All others were either non-pathogens or at best could be described as low-grade pathogens or opportunist-type organisms.

Insects from 4 accessions included bacteria belonging to the Bacillus cereus group. From one of these accessions Bacillus thuringiensis was isolated from Melanoplus bivittatus. From 2 of these accessions, crystalliferous sporeformers, yet to be identified, were isolated from Galleria mellonella, and Ostrinia nubilalis. From the fourth, Bacillus cereus was isolated from larvae of Anthonomus sp.

Heliothis zea eggs and hatching larvae suspected of being infected with Serratia marcescens were submitted by the Winter Vegetables Insects Investigations, Charleston. Cultural and biochemical studies established that the bacterium isolated was S. marcescens Bizio.

4. Studies on bacteria isolated from cereal leaf beetle. During the year extensive studies on the cereal leaf beetle were continued. The studies were carried on cooperatively with the staff of the Biological Control Laboratory, Plant Pest Control Division, Niles, Mich., to determine the cause of high mortalities in laboratory colonies of the beetle. Samples of affected insects and cultures obtained from apparently sick specimens were received at this laboratory for diagnosis the previous year. Examination of the insects had revealed no organisms which could be held definitely responsible. However, a total of 34 bacterial cultures were reisolated from the original 5 cultures submitted. Sixteen of the isolates proved pathogenic to wax moth larvae by injection. Transfers of the 16 isolates were then tested at the Niles laboratory by feeding apparently healthy beetle larvae on oat plants treated with the various isolates. Although their data showed higher mortalities in all but three of the treated groups compared to controls, it did not appear too significant in view of the high dosages and the low numbers of insects used in the test.

Antibiotic sensitivity tests conducted, indicated that only 5 different bacteria were involved and that Neomycin sulfate would inhibit the entire group.

In November, 1967, John Thompson of our staff visited the Niles laboratory and made microscopic examinations of a number of specimens of all stages of the insect but found no single organism that could be held responsible. Following his suggestion they lowered the relative humidity in the rearing chambers and employed Neomycin sulfate to curb bacterial contamination. Although Neomycin improved larval survival to the 4th instar, neither suggestion has fully provided the answer to the problem. It would appear that none of the bacterial isolates tested is the primary cause but that other predisposing factors are involved thus placing the insect under stress so that opportunist-type organisms can take over. In this regard the use of hydroponics to grow the insect fodder is seriously suspected as the stress agent.

5. Studies on Bacillus popilliae Dutky. Dried blood films prepared from Cochliotus melolonthoides injected with Type A Milky disease spores from Japanese beetle were received for confirmation of infection from the Tanganyika Planting Co., Ltd., Moshi, Tanzania. From 2 of the 7 slides submitted, a suspension of 0.4 ml containing a total of only  $20 \times 10^6$  spores was prepared. Each of 19 3rd instar Japanese beetle larvae injected with this material received a dosage of  $3 \times 10^7$  spores. Fourteen of the 15 larvae surviving the second day following injection became infected by the 9th day. All larvae showed typical symptoms and blood from all individuals examined revealed typical Type A spores of Bacillus popilliae.

6. Studies on *Drosophila melanogaster*. Personnel concerned with mass-rearing *Drosophila melanogaster* at the Vegetable and Ornamental Insect Investigations at Beltsville, have recently experienced unusually low yields in their colonies. In studies to obtain an aseptic colony, surface sterilization of the eggs was necessary. Although the eggs withstand sodium hypochlorite treatment, difficulties arose in obtaining a large quantity of eggs of the same age free of hatching larvae. When hypochlorite-treated eggs were dispensed in tubes containing tryptose-phosphate agar, tubes containing larvae that had hatched before the sterilization procedure was started, showed bacterial growth. Two different bacterial isolates were obtained from such tubes, one of which proved pathogenic to young *Drosophila* larvae.
7. Other bacterial infections. Other infections involved either nondescript types of bacteria not conforming to published descriptions of known species, or as yet have not been identified. Pure cultures of a number of these organisms were obtained, many of which were tested for pathogenicity by feeding and injection. Bacteria of these types were isolated from Mexican bean beetle, the gloomy scale, catalpa sphinx moth, *Diabrotica* sp., and from the fall armyworm and its parasite, *Camponotus perdistinctus*.
8. Undetermined infections. Accessions containing insects in which no evidence of disease could be detected or confirmed comprised 23 percent of the accessions received during the year. Insects examined which revealed no disease included the following; southwestern corn borer eggs and adults; melon aphids; brown soft scale; puparia of *Drosophila melanogaster*; cereal leaf beetle larvae, pupae, and adults; nymphs and adults of *Aphis fabae*; and larvae of *Anthonomus grandis*.

#### F. General Subjects

1. Biology of the stilt bug. Preliminary to any development and use of microbial pathogens is the control of insect pests, some information concerning the life cycle of the particular insect must be obtained. A study during the summers of 1965-67 of the stilt bug, *Jalysus spinosus*, revealed that this insect feeds on tobacco and tomato leaves and flowers by puncturing the leaf parts with its proboscis and sucking the fluids of the plant. Adults were observed by the middle of June around young tobacco plants. By the last week in June the adults had commenced to mate. Egg laying began by the second week in July with each female depositing 8 to 15 eggs every 24 hours. Total egg production/female approximated 200 to 400.

After four days the nymphs emerged, reaching maturity in 5 weeks. As late as the first week in September, first and second instar stilt bugs were observed. The insect overwinters under rotting debris in the egg stage.

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Area No. 18. Insect Physiology and Mode of Action of  
Insecticides and their Metabolites

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-years FY 1968				
	Research Problem Area				
	204	207	210	706	Total
Maryland (Beltsville)	2.7	6.7	1.9	1.2	12.5
North Dakota	0.8	2.0	2.2	2.5	7.5
Texas	0.4	1.6			2.0
Total	3.9	10.3	4.1	3.7	22.0

Intramural program is supplemented by extramural support representing P.L. 480 funds in one country representing 57,484 U. S. Dollars equivalent.

## Problems and Objectives

Resistance to insecticides by insects has emphasized the need for information on the mode of action and metabolism of insecticides in insects and the mechanisms of the resistance. Basic research in insect biochemistry and physiology is needed to provide a better understanding of the biochemical and physiological systems which regulate insect growth, metamorphosis, reproduction and diapause, and the chemistry and action of the hormones which mediate these systems. Such research is essential to the development of new methods of effective insect control which are safer and more selective in their action and will reduce or avoid problems of insecticide residues in or on food and forage crops and pollution of the environment. More basic information on the response of insects to light, sound, food, and sex attractants is needed.

Major objectives of the research are to develop basic knowledge of the:

1. Biochemistry and physiology of lipids in insects, insect hormones and nutrition.
2. Metabolism of insecticides and other compounds in insects.
3. Physiological processes specific to insects.
4. Physiology of insect growth and development.
5. Insect biorythms.

## Progress - USDA and Cooperative Programs

### Insect Physiology Pioneering Research Laboratory

#### A. Biochemistry and Physiology of Lipids in Insects (2.7 SMY)

1. Desmosterol as a common intermediate in the conversion of a number of C<sub>28</sub> and C<sub>29</sub> plant sterols to cholesterol by the tobacco hornworm. In addition to  $\beta$ -sitosterol, a number of other plant sterols, including stigmasterol, campesterol, fucosterol, 24-methylene-cholesterol, 22,23-dihydrobrassicasterol, and brassicasterol are converted to cholesterol in the tobacco hornworm. When insects were reared on larval diets containing each of these sterols, cholesterol was the major sterol (70%) present in the insect tissues, indicating that the hornworm larva is capable of dealkylating  $\alpha$ - or  $\beta$ -methyl,  $\alpha$ -ethyl, methylene, and ethylidene groups from the C-24 position of the sterol side chain and can also saturate the  $\Delta^{22}$ -double bond. Larvae on diets containing either brassicasterol or dihydrobrassicasterol grew and developed slowly whereas the growth rate of insects fed campesterol, stigmasterol, 24-methylenecholesterol, or fucosterol was equivalent to that of controls fed  $\beta$ -sitosterol.

When 20,25-diazacholesterol, which blocks the conversion of desmosterol to cholesterol in this insect, was added to the diets

in combination with each of the test sterols, severe retardation of growth and development occurred. This effect was most severe in insects fed diets containing brassicasterol or dihydrobrassicasterol plus the diazasterol. In addition, the diazasterol brought about abnormalities in development such as formation of prepupal-pupal intermediates, indicating that the azasterols may in part be effecting its inhibitory action by interfering with certain endocrine-mediated processes in the hornworm. In all cases, there was an accumulation of desmosterol and a concurrent decrease in cholesterol in the sterols isolated from insects fed a phytosterol plus diazasterol. Apparently, the  $\Delta^{24}$ -sterol reductase enzyme system, which is blocked by the diazcholesterol, is involved in the formation of cholesterol from all these sterols, indicating a similarity in the terminal step of the conversion of these phytosterols to cholesterol by the hornworm.

24- $\beta$ -Alkyls of brassicasterol and dihydrobrassicasterol appeared to be less efficiently dealkylated than the 24- $\alpha$ -alkyls of campesterol,  $\beta$ -sitosterol and stigmasterol. This may be related to an adaptation of the sterol complex available to this insect from tobacco, in which stigmasterol and  $\beta$ -sitosterol are the predominant sterols. There was a more efficient utilization of fucosterol and 24-methylenecholesterol than of any other sterols in the absence of diazasterol and feeding these two sterols in combination with the diazasterol resulted in the greatest accumulation of desmosterol and the last unchanged residual dietary sterol. This suggests that these two sterols may be normal intermediates in the dealkylation scheme. Further detailed information on the dealkylation mechanism is a requisite both to an understanding of the comparative sterol biochemistry of phytophagous insects and their host plants and to a rational approach to the development of chemicals that selectively disrupt sterol dealkylation in plant-feeding insects. (207)

## 2. Conversion of cholestanone to cholestanol by the house fly.

Cholestanol and cholestanone have both been reported to be steroid antimetabolites for the oriental house fly while the steroid ketone cholestenone was reported to serve as a sole source of dietary sterol for the growth and development of this insect. This is an interesting relationship as in certain vertebrates cholestenone is metabolized to cholestanol with cholestanone as an intermediate in the transformation. Nutritional and biochemical studies were undertaken to study the action and metabolic fate of these steroids in the house fly.

When larvae of the house fly were reared aseptically on a semi-defined diet, they failed to develop when either cholestenone, cholestanone, or cholestanol was used as the sole source of dietary steroid. However, when these three steroids were tested for "sparing" activity (in combination with a subminimal quantity

of cholesterol), cholestanone and cholestanol were 66 and 79%, respectively, as effective as an optimum concentration of cholesterol. Under the same conditions, cholestenone showed only low biological activity (11%).

Biochemical studies with 4-<sup>14</sup>C-cholestanone indicate that the utilization of this steroid in the house fly larvae proceeds through its partial conversion to cholestanol. The identity of cholestanol as a metabolite of cholestanone was established by radiotracer techniques, gas-liquid chromatographic analysis, and by isolation of crystalline cholestanol from house fly pupae reared aseptically on a semidefined diet containing cholestanone plus a subminimal amount of cholesterol. Using reverse isotope dilution techniques, no significant conversion of <sup>14</sup>C-cholestanone to cholesterol was detected. In studies with adult insects, both males and females converted injected <sup>14</sup>C-cholestanone to cholestanol.

The house fly then differs from the oriental house fly in that rather than being sterol antagonists, both cholestanone and cholestanol will fulfill in part the house fly's dietary sterol requirements. Also, cholestenone has, at most, only slight growth promoting activity for the house fly. A decision as to whether or not such striking differences actually exist in the steroid biochemistry of these two closely related insects must await further nutritional and biochemical experimentation with the oriental house fly. (706)

#### B. Insect Hormones (4.3 SMY)

1. Juvenile hormone activity of insecticide synergists and synthetic compounds. In an attempt to increase the juvenile hormone activity of 10,11-epoxyfarnesenic acid methyl ester by co-application of hormones and piperonyl butoxide it was discovered that the piperonyl butoxide alone produced juvenile hormone activity. Several other synergists were examined for activity on pupae of Tenebrio and the most active was sesamex which produced second pupae with dosages as low as 0.25 µg. Piperonyl butoxide causes retention of pupal genitalia with 10.0 µg. These compounds were also effective when applied to milkweed bug nymphs. Sesoxane and piperonyl butoxide caused molting to nymphal-adult intermediates at 1 µg. A new synergist, Niagara 16388 (propyl 2-propynyl phenylphosphonate), produced pupal-adult intermediates with Tenebrio at 0.5 µg, but was completely ineffective on milkweed bugs. Thus, this latter compound appears to have a high degree of species specificity.

Since two of these active synergists were methylenedioxyaromatic derivatives, piperonyl farnesyl ether was synthesized and tested for activity. This ether produced pupal-adult intermediates in Tenebrio at 100 µg but was effective in producing nymphal-adult

intermediates in milkweed bugs at 1  $\mu$ g. Epoxidation of the terminal double bond of the farnesyl moiety increased activity approximately ten fold in both assays.

The potential of insect juvenile hormones as agents for insect control has been the subject of considerable speculation. To date, however, most of the compounds of significant activity have been available only as laboratory tools, as the cost of synthesis of these in quantities adequate for field studies has been prohibitive. The present study has revealed several compounds of high biological activity which are already available in commercial quantities. (Multiple RPAs)

2.  $\alpha$ -Ecdysone and 20-hydroxyecdysone in bracken fern (*Pteridium aquilinum*). Recent reports that plants yield in extremely large quantities steroids that are similar or related to the insect molting hormones prompted examination of certain primitive plants. Ferns, which are widely distributed and are relatively immune to insect attack were considered ideal subjects for such studies.

A sufficient quantity of the dry bracken (4 kg) was collected in mid-January, and the pinnae were separated from the plant and pulverized to a powder. The techniques used to isolate and purify the extracted biologically active components were similar to those used to isolate the ecdysones from insect sources.

The concentrate, after adsorption chromatography, was subjected to a countercurrent distribution system of cyclohexane-butanol-water 5:5:10, and in this solvent system two major peaks were detected by the house fly assay. Preparative thin-layer chromatography (TLC) of the pooled fractions representing the faster moving less polar peak in countercurrent distribution, gave a major zone with an  $R_f$  similar to  $\alpha$ -ecdysone and three additional minor zones with activity as well. The zone corresponding to  $\alpha$ -ecdysone yielded 1.8 mg of crystalline material. Preparative TLC of the more polar material from countercurrent distribution gave a zone with an  $R_f$  value similar to that of 20-hydroxyecdysone. This zone, which contained the only active principal on the plate yielded 4.0 mg of crystalline material. Mass spectral analysis of the crystalline apolar and polar compounds from the fern indicated molecular weights of 464 and 480, and gave fragmentation patterns identical to those of  $\alpha$ -ecdysone and 20-hydroxyecdysone, respectively. Their identity was further confirmed by other physical properties (UV, IR and NMR spectra and melting points). Bioassay of the crystalline hormones from bracken showed these to be equal in activity to the authentic  $\alpha$ -ecdysone and 20-hydroxyecdysone isolated from insects.

Bracken, then, is the first plant found to contain both major insect ecdysones and is the first known plant source of  $\alpha$ -ecdysone.

The presence in bracken of  $\alpha$ -ecdysone and 20-hydroxyecdysone suggests similar metabolic pathways for steroids in the insect and in this fern. The identification of the three biologically active unknowns could well provide us with information on the biosynthesis and metabolism of the ecdysones in insects.

We have detected molting hormone activity in the crude extracts of pinnae from the four other species of ferns so far examined; the sensitive fern (Onoclea sensibilis L.), Christmas fern (Polystichum acrostichoides (Michx.) Schott), common polypody (Polypodium virginianum L.), and cinnamon fern (Osmunda cinnamomea L.). The titer, however, varied considerably among the different species of ferns which could reflect a qualitative, as well as a quantitative difference in the ecdysones present. The ferns thus could serve as a rich source for the molting hormones. (Multiple RPAs)

3. Effects of ecdysones and analogs on insect development and reproduction. To assess the role of the ecdysones and related steroids as plant protectants as well as their potential as insect control agents several natural ecdysones and synthetic analogs were fed to five species of insects. Certain of the synthetic analogs, particularly  $\Delta^7$ -5 $\beta$ -cholestene-2 $\beta$ ,3 $\beta$ ,14 $\alpha$ -triol-6-one ("Triol"), were potent inhibitors of larval growth and development in several species of insects when ingested. Considerable differences in species susceptibility to the "Triol" was noted; yellow fever mosquito larva was inhibited by less than a ppm, house fly larvae at 25-50 ppm while the immature firebrat was unaffected at 10,000 ppm. Certain natural ecdysones, including the insect ecdysone, 20-hydroxyecdysone, were generally found to be inactive or considerably less active than the synthetic analogs. The plant ecdysone ponasterone A, however, was more inhibitory for confused flour beetle larvae than the most active synthetic analog, the "Triol".

When fed to the adult housefly the synthetic "Triol" and the naturally occurring ecdysones, 20-hydroxyecdysone and ponasterone A, inhibited ovarian maturation and egg production. The "Triol" also inhibited reproduction in the female confused flour beetle. Preliminary tests indicate that with both species the major effect of these steroids is on the female reproductive system.

The inhibitory effects of these steroids appears to be related to their hormonal activity as evidenced by their "anti-gonadotropic" activity in adults, their interference with normal molting and morphogenesis in immature insects and the finding that the hormonally inactive (molting hormone assay) 5 $\alpha$ -isomers of the analogs were not inhibitory.

Aside from the possible role of the ecdysone in insect host-plant interactions these results point to practical implications and

potential applications for these steroids. Certain synthetic analogs with only minimum structural features inhibited larval growth and development in a number of insects. The finding that a synthetic analog and certain natural ecdysones, when fed, inhibit ovarian development in insects points to these compounds as models for the development of safe and specific insect anti-fertility agents. (Multiple RPAs)

4. Effects of ecdysones and analogs on ovarian development and reproduction in the house fly. The "anti-gonadotropic" effect of certain ecdysones and analogs on the adult female house fly suggests that these steroids may serve as regulators of the reproductive processes of insects. Studies on structure and biological activity were undertaken to further evaluate these compounds as insect antifertility agents.

The naturally occurring ecdysones (20-hydroxyecdysone,  $\alpha$ -ecdysone, inokosterone, and ponasterone A) when fed in an artificial diet were found to be less active as inhibitors of ovarian development than the synthetic ecdysone analog,  $\Delta^7$ -5 $\beta$ -cholestene-2 $\beta$ ,3 $\beta$ ,14 $\alpha$ -triol-6-one ("Triol"). The natural ecdysones completely inhibited ovarian growth (terminal oocyte less than 0.3 mm) at concentrations of 0.50 to 0.75% whereas only 0.1% of the "Triol" was required to exert the same effect. At concentrations as low as 0.05% the "Triol" was found to reduce the progeny by 77% and the inhibitory effects of the "Triol" were only slightly reversed in long term studies. The corresponding 5 $\alpha$ -analog of the "Triol" ( $\Delta^7$ -5 $\alpha$ -cholestene-2 $\beta$ ,3 $\beta$ ,14 $\alpha$ -triol-6-one) was inactive even when fed at concentrations as high as 1% indicating a high degree of specificity in relation to the configuration of the A/B ring.

The least inhibitory natural ecdysone, 20-hydroxyecdysone, and the "Triol" were compared by injection in emulsions and the natural insect ecdysone was found to be more active than the "Triol" suggesting that the observed differences in activity may be in part due to differences in intestinal absorption and/or metabolism. Since the "Triol" is more apolar than the natural ecdysones, three less polar derivatives of the 20-hydroxyecdysone (the diacetone, triacetate and tetra-trimethylsilyl ether) were prepared and tested. The tetra-trimethylsilyl ether of 20-hydroxyecdysone was nearly 10 times as active as the parent compound by ingestion and at 0.1% caused inhibition comparable to that found for the "Triol" and its corresponding derivative at this same concentration. Interestingly, the silyl ether derivatives of 20-hydroxyecdysone and the "Triol" were found to penetrate the cuticle and thus were inhibitory when applied topically. The diacetone and triacetyl derivatives were less active than the parent compound.

The above results, as well as molting hormone assays have shown that either the number or the position of the hydroxyl groups

present in the ecdysone molecule affect the biological activity of these steroids. A number of insecticide synergists have been reported to affect microsomal hydroxylation in insects and certain of these were tested as to their effect on the inhibitory activity of the "Triol". Feeding the "Triol" in combination with synergists such as piperonyl butoxide or sesoxane (1:10) was found to enhance ovarian inhibition 5 to 10 fold and to drastically interfere with reproduction. These synergists also have recently been shown to have juvenile hormone activity. Studies to determine how these synergists and other juvenile hormone mimics enhance the inhibitory activity of ecdysone analogs may provide us with information on the interaction of these two types of hormonally active compounds as well as techniques for enhancing the antifertility activity of the ecdysones and analogs. (706)

#### Metabolism and Radiation Research Laboratory

##### A. Fate and Physiological Effects of Chemosterilants and Other Compounds (1.0 SMY)

1. Automated analysis of total and free aziridine. An automatic colorimetric method based on the Epstein procedure was developed for the determination of total aziridine in 25 aziridine--containing candidate insect chemosterilants by using the Autoanalyzer. Methanolic solutions of these compounds can be analyzed at the rate of 10 to 70 samples per hour. The precision and reproducibility of data obtained with this method are excellent. The range of concentration that can be analyzed is from less than 1 µg/ml to more than 1000 µg/ml. (Multiple RPAs)

2. Residue analysis of a screw-worm chemosterilant. Residues of N,N-tetramethylenebis-1-aziridinecarboxamide were determined on Phormia regina by the automated aziridine analysis. Approximately one-third of the original dosage of this chemical remained after a three-week exposure. (210)

3. Disappearance of a phosphoramidate chemosterilant in the boll weevil. The cotton boll weevil, Anthonomus grandis (Boheman), was treated by topical application, tarsal contact, and injection with N,N,N',N'-tetramethyl-p-piperidinophosphonic diamide. Gas chromatographic analysis of the residues remaining in or on the boll weevil at various times after treatment showed that the greater part of the residues had disappeared after 24 hours, regardless of the method of treatment. The rate of disappearance of the parent compound from the males injected with 20 µg of the compound was extremely rapid; only 20% of the original dose remained one hour after injection. (207)

##### B. Physiological Processes Specific to Insects (3.5 SMY)

1. Effect of various biogenic amines on the activity of the foregut of the cockroach, *Blaberus giganteus*. A differential transformer displacement transducer was used to record the activity of the visceral muscle of *Blaberus giganteus* (L.) with a high degree of sensitivity. The denervated foregut proved to have myogenic properties with certain specific pacemaker regions. Nevertheless, stimulation of the oesophageal nerve innervating the region of the crop caused a slow type of graded contraction in the longitudinal muscles. Preparations of both the innervated and the denervated foregut were sensitive to 5-hydroxytryptamine ( $10^{-7}$  M), noradrenaline ( $10^{-6}$  M), and acetylcholine ( $5 \times 10^{-6}$  M). Also, Factor S, a biogenic amine of uncertain structure found in arthropods, caused an excitatory response in innervated foreguts and an inhibitory response in denervated foreguts. Denervated foreguts had a greater sensitivity to L-glutamic acid than innervated preparations. (706)

2. Esterases and phosphatases in the gastric secretion of the cockroach, *Periplaneta americana*. Zone electrophoretic analysis of the gastric secretion of the American cockroach revealed the presence of six carboxylic esterases. Two of these enzymes are resistant to  $10^{-4}$  M dichlorvos and one, E6, is localized in the epithelium of the gastric ceca and the midgut. A procedure was developed for the purification of esterase E6 which yielded a single zone after starch gel electrophoresis. The purified enzyme readily hydrolyzed  $C^{14}$ -labeled tripalmitin to free fatty acid, diglyceride, and monoglyceride and l-naphthyl acetate to free l-naphthyl and acetic acid, and 86% of both the estralytic and lipolytic activity was inhibited by  $10^{-3}$  M dichlorvos. Both the calcium and manganous ions served as activators in triglyceride hydrolysis.

Also, three phosphatases were isolated from the gastric fluid; one having an alkaline pH, the second with an acid pH, and a third with a pH optimum near 7. The acid phosphatase was inhibited by  $10^{-3}$  M manganous ion; the neutral phosphatase showed a marked increase in activity in the presence of the same cation.

The role of these hydrolyses in the normal functioning of the insect and in the metabolism and mode of action of insecticides is under investigation. (706)

3. The distribution of Factor S in the cockroach, *P. americana* and its role in stress paralysis. Bioassay was used to determine that the ventral nerve cord and head of the cockroach, *P. americana* (L.), contained the highest concentrations of Factor S; the body and legs contained lesser amounts. A two-fold increase in the titer of Factor S was observed by spectrofluorometry and by bioassay in insects subjected to four hours of mechanical stress compared with the amounts in unstimulated insects. House flies

injected with 20 µg of the active residue were immediately paralyzed: 85% were unable to right themselves within three hours; after 24 hours, 60% were dead, and 15% of the survivors had a marked impairment in motor coordination. A substance that caused much the same biological response was found in perfusates from electrically stimulated isolated ventral nerve cords of cockroaches. (706)

4. Neuromuscular substances in insects. Work is continuing in the area of isolation and characterization of the neuroactive substances from several arthropods. Techniques were developed to extract and isolate these substances on a several kilogram scale of tissue. Extracts were obtained from the crayfish and cricket. Assay of the substances on the isolated nerve cord of the cockroach (*P. americana*) showed that the purification has provided a substance that has a specific activity of  $6.4 \times 10^{10}$  standard responses per crayfish or  $2.56 \times 10^6$  responses/mg of crayfish. Small quantities of the substance are available, and chemical analysis is underway.

Infrared and nuclear magnetic resonance spectra of the substance suggest that the substance is a simple organic molecule. Mass spectrometry did not yield much information because the substance is not volatile and pyrolyzes. Derivatives were formed that will possibly allow mass spectral analysis. (706)

5. Hydrocarbon synthesis in the cockroach. Studies of hydrocarbon synthesis in the integument and fat body in vitro showed that the integument was capable of hydrocarbon synthesis from acetate and palmitate. No hydrocarbon synthesis was observed in the fat body. Cytological examination of the tissues showed that oenocytes were present in the fat body and possibly in the integument. The lack of hydrocarbon synthesis in the fat body indicates that the oenocytes do not synthesize hydrocarbons and that the hydrocarbon synthesis observed in the integument must be due to the epidermal cells. (706)

6. Isolation and characterization of insect esterases. The action of detoxifying esterases may be involved in species specificity in poisoning by organophosphates and in the acquired resistance of some insects to certain of these insecticides. In addition, the inhibition of these enzymes may account for insecticide toxicity under conditions in which no impairment of neural function has occurred. Recent experiments have indicated a possible role of esterases in lipid metabolism in the fat body and hemolymph.

Esterases are present in the cockroach midgut and gastric ceca, fat body, and hemolymph which are capable of hydrolyzing long-chain triglycerides. Recent experiments in this laboratory on

lipid synthesis and release by the fat body have indicated a possible role of esterases in lipid metabolism in the fat body and hemolymph.

Esterases from the midgut and gastric ceca of the cockroach were purified by the sequence of acetone powder, ammonium sulfate precipitation, heat, and ion exchange chromatography, and starch gel electrophoresis. Two esterases were separated on the chromatography column which migrated similarly on starch gel electrophoresis. Phosphatase activity was less than 0.4% of the esterase activity based on their hydrolysis of naphthyl acetate and naphthyl phosphate. They were inhibited by DDVP (dimethyldichlorovinyl phosphate, a cholinesterase inhibitor), and this inhibition of naphthyl acetate hydrolytic activity was partially reversed by magnesium ions. The enzymes were also inhibited by DFP (diisopropyl fluoro phosphate) but not by eserine. Additional studies with Sephadex column chromatography on these enzymes are in progress. (706)

7. Studies on house fly ejaculatory duct. A substance in the seminal fluid produced by the male house fly ejaculatory duct has been shown to induce a mating avoidance response by the treated females. A series of histochemical analyses indicated that secretory activity begins as early as the late pupal stage and persists throughout the reproductive life of the insect. This activity is evidenced by the abundant RNA synthesis that occurred only in cells of the duct that produced the secretion. The area surrounding the lumen of the ejaculatory duct where the secretion is stored contained a heavy concentration of proteinaceous material. This region also yielded a positive acid-Schiff reaction, suggesting that the duct secretion may be a complexed protein-carbohydrate substance such as glycoprotein.

Treatment of the duct tissue with several proteolytic enzymes revealed that protein stainability was reduced in the cytoplasm of the secretory cells and in the storage area only by pronase, which is relatively unspecific in its action. Pepsin digestion did not hydrolyze the stored secretory material although the staining intensity of protein within the cell cytoplasm was reduced.

The accessory fluid was also found to exist in a stainable concentration within the copulatory vesicles of females examined immediately after mating. However, it is not present at this site 24 hours after mating. Additional studies using radioactive metabolites are being conducted in an effort to trace the route of the accessory material to a possible target organ. (706)

8. The monocoitic substance. Studies were undertaken to isolate and characterize the substance present in the copulatory duct of the male house fly, Musca domestica (L.), which prevents females

from mating a second time. An extract of the copulatory ducts, when injected into virgin females, prevented them from mating. Extracts were prepared from whole male house flies by extracting with distilled water or 95% ethanol. The initial extract was very toxic, and it was necessary to dialyze it to remove excess salts and other low molecular weight substances before it could be bioassayed.

Attempts at purification of the active substance present in the extracts included the use of techniques such as heating, dialysis, gel filtration chromatography on Sephadex, acetone precipitation, and the removal of nonpolar substances with organic solvents. The majority of the activity remained in the dialysis tube when the extracts were dialyzed against distilled water. The activity eluted as a low molecular weight substance when the extracts were chromatographed on Sephadex and eluted with water. The active substance was soluble in 95% ethanol, methanol, and water, but it was insoluble in absolute ethanol, acetone, ether, and chloroform. The active substance was relatively unstable but retained about 50% of its activity after 30 days when stored dry under nitrogen at -20°C. Active extracts that prevented mating of female house flies were obtained from the male house fly, screw-worm fly, and black blowfly. An extract of Aedes aegypti did not inhibit mating of female house flies. (706)

### C. Insect Growth and Development (3.0 SMY)

1. Oöstatic hormone studies. An oöstatic hormone is produced by house flies with eggs in stages 4 through 10. This hormone suppresses the development rate of stages 2 to 4 eggs; complete inhibition of the second gonotropic cycle at stage 4 occurs when mature eggs from the first cycle are retained.

The hormone was extracted from whole mature female flies and from mature ovaries. When the extracts were injected into 24-hour-old females, the first gonotropic cycle was inhibited. Studies are in progress to purify and identify this hormone. Some extracts were active when injected at 10 µg or less.

The oöstatic hormone seems to act like the corpus allatum. In some way, the presence of the oostatic hormone prevents the release of juvenile hormone; by so doing, vitellogenesis does not occur.

This hormone has a very specific mode of action and has control potential. A compound that can inhibit the release of juvenile hormone can stop ovarian development and prevent mating in the house fly. This hormone should be effective on other dipterous insects. (706)

2. Endocrine regulation of reproduction in the house fly. We demonstrated the role of the corpus allatum on mating and ovarian development in the house fly. When females were allatectomized at an age of 48 hours or younger, both ovarian development and mating were inhibited. Allatectomy of females 60 hours of age or older had no effect on mating or ovarian development. Topical application of 2.5 µg of synthetic juvenile hormone stimulated both mating and ovarian development in allatectomized females. (706)

3. Synthetic juvenile hormone studies. Studies are in progress to determine the effectiveness of synthetic juvenile hormone to sterilize female house flies. We found that the topical application of 20 µg of T,T, 10,11 epoxy farnesenic acid methyl ester to gravid females resulted in 20% egg viability in both the first and second gonotrophic cycles. (706)

4. Development of a tissue culture medium. Modifications of the M-14 chemically defined nutrient medium were made which retarded the precipitation of calcium phosphate in long-term cultures. In addition, a new M-20 medium that contains fetal calf serum was devised. This medium is extremely stable and will maintain cockroach cell cultures indefinitely. (706)

5. The maintenance of insect cell cultures. Monolayer cell cultures from four insects: Leucophaea maderae, P. americana, Melanoplus differentialis, and M. domestica, are being maintained and are available for experimentation. Some of these cultures are over a year old.

Short-term organ cultures of tissues from L. maderae and the tobacco hornworm, Manduca sexta (Johannson), are being maintained and used in endocrine studies.

The RIM-10 strain of cells from Antheraea eucalypti (Scott) are also being maintained for use in developing methods and techniques for work with insect cell cultures. (Multiple RPAs)

6. Endocrine studies in vitro. Studies were conducted on the effects of the various ecdysones and ecdysone analogs on the development of two types of organ cultures: the development of rectal pads during metamorphosis in M. sexta and the regeneration of nerve and the production of exoskeleton in cultures of cockroach leg regenerates. β-Ecdysone added to the culture medium stimulated the differentiation of rectal pads and the secretion of exoskeletal materials in these cultures. (207)

7. Screening systems for biologically active compounds. Two systems using in vitro culture methods were devised to test the results of biologically active compounds at the cellular level. The first of these employed sperm from the spermathecae of

P. regina. Compounds to be tested for sperm inactivation effects were added to the nutrient medium in which sperm-filled spermathecae were crushed. The results were read with a phase contrast microscope.

The second system employed cultures of dispersed tissue into which test compounds were introduced. The effect of the test compounds on the uptake by the cells of tritiated thymidine and uridine and  $C^{14}$ -labeled amino acids was determined by liquid scintillation. (706)

8. Photoperiodic induction of diapause in *Manduca sexta* (Johannson). Laboratory studies on the photoperiodic control of insect diapause were conducted by using tobacco hornworms reared on artificial diet. The study was undertaken to gain basic information on the physiology of insect photoperiodism.

Diapause, which occurred in the pupal stage, was readily induced by rearing the eggs and larvae in daily photoperiods of 13 hours or less; longer photoperiods prevented diapause. All individuals entered diapause when reared in a 12-hour photoperiod and remained in the arrested state longer than insects reared in shorter photoperiods.

Reception and integration of the photoperiodic stimulus began during late embryonic development and continued during the process of larval growth. The photoperiod produced maximum response during the periods when food uptake and growth rate were maximal.

Only three or four consecutive daily photoperiods of 12 hours were required to induce diapause in some individuals; 12 cycles produced 100% response. The diapause-inducing effect of 12-hour photoperiods persisted in aperiodic continuous light but was readily reversed by photoperiods having 15 hours of light and 9 hours of darkness per daily cycle (designated LD 15:9). Only two or three LD 15:9 cycles were required to nullify the diapause-inducing effect of 15 to 18 LD 12:12 cycles.

Diapause-inducing LD 12:12 cycles were converted to a diapause-preventing regimen by introducing light (6 minutes) one and one-half to three hours after the beginning of the dark phase. However, this "diapause-preventing light break" was, in turn, rendered ineffective if the subsequent 9-hour dark period was interrupted with light. (207)

#### D. Cytochemistry and Cytophysiology of Lipid and Nucleotide Metabolism of the Animal Cell

1. Nucleic acid metabolism in developing ovaries. Nucleic acids were extracted from ovaries dissected from prepupae and adults of the wax moth, Galleria mellonella. The amounts of DNA and RNA,

as estimated for the fresh weight of the tissue, were higher in prepupal gonads than in imaginal gonads. (314)

2. Hormonal control of oöcyte growth. Prepupae of G. mellonella were ligated behind the prothoracic segment, and the synthesis of DNA in their gonads was investigated by means of autoradiography. The number of nuclei labelled with  $H^3$ -thymidine decreased sharply after depriving the prepupae of a normal hormone flow. (314)

3. Ultrastructure of the developing ovary. Electron microscope studies were made on the ultrastructure of normally developing prepupal ovaries and of those experimentally deprived of hormonal inflow. The lack of normal hormone supplies induced ultrastructural changes. (314)

#### Pesticide Chemicals Research Branch

##### A. Insect Biorythms (1.5 SMY)

##### 1. Photoperiodism and diapause.

a. Action spectrum for breaking diapause. The portion of the visible spectrum most effective in breaking diapause of oak silkworm pupae (Antheraea pernyi) and codling moth larvae (Carpocapsa pomonella) lies between 400 and 520 nm. Less than  $1 \mu\text{w}/\text{cm}^2 \times \text{nm}$  in this region is required to break diapause. Since homogenates of oak silkworm brains and of codling moth heads exhibit a relatively high absorption at these wavelengths, materials isolated from the homogenates are being compared with commercial compounds of similar absorption characteristics and with naturally occurring substances. This work is being done in cooperation with the Market Quality Research Division. Modifications of electrophoretic techniques which minimize oxidation of naturally occurring pigments have been developed for this purpose.

The instruments used to obtain absorption spectra are a commercial microspectrophotometer and a similar instrument constructed in the Market Quality Research Division. Studies were carried out on the brains of diapausing oak silkworm pupae in the sensitive area delineated by Williams, in homogenates of the heads of larval codling moths, in intact eggs of the corn earworm (Heliothis zea), the tobacco budworm (H. virescens), and the pink bollworm (Pectinophora gossypiella). Each of these insects is subject to photoperiodic control of diapause. (Multiple RPAs)

##### 2. Manipulating photoperiods to damage insects.

a. Diapause studies. The possibility of manipulating photoperiods to upset the normal adaptation of insects to the

environment was explored in the laboratory and outdoors. The response curve for breaking the pupal diapause of A. pernyi and the larval diapause of C. pomonella was determined by exposing the insects in a series of light-tight chambers at  $25^{\circ} \pm 1.5^{\circ}\text{C}$  to a 16-hr day followed by 15-minute light breaks during different portions of the night. Peaks of sensitivity to breaking diapause occurred 2 hr after dark and 2 hr before dawn. By contrast a light break 3 hr before dawn significantly reduced the breaking of diapause.

Similar results were obtained when different lots of the same insects were exposed outdoors in Beltsville, Md., starting in late February, to natural light and temperature conditions supplemented by 1-hr light breaks (fluorescent lights) at night. These results are promising, but more work (particularly under outdoor conditions) is needed to determine the role of photoperiod manipulation in insect control. (Multiple RPAs)

b. Growth studies. Cockroaches (Leucophaea maderae) 1-7 days old, were placed in dishes with food and water in closed chambers ( $25^{\circ} \pm 1.5^{\circ}\text{C}$ ). The insects were exposed for 180 days to 5 experimental light regimens: (a) constant darkness (DD); (b) constant light (LL); (c) LD 12:12; (d) LD 12:12 with 15-minute light breaks 2 hr after the end of the light period and 2 hr before the beginning of the light period; and (e) LD 12:12 cycled with LD 18:6 every 4 days. Growth rates were exponential during the period of study. Conditions (d) and (e) significantly (t test) reduced the growth rate, possibly because the circadian rhythms involved were entrained at different rates, thus upsetting metabolic processes. A similar reduction in growth rate and a high mortality was obtained when H. virescens was reared under condition (e). (706)

c. Effects of temperature on insects. A specially-designed temperature gradient block has been utilized to study the effects of temperature on breaking pupal diapause in the oak silkworm and larval diapause of the codling moth. Also, the rates of development of H. zea and H. virescens are being compared in the range between  $13^{\circ}$  and  $35^{\circ}\text{C}$  with long and short day exposures. (207)

### 3. Biochemical studies.

a. Microsomal activity. Activity in vivo of the microsomal fraction of insect tissue may be responsible not only for susceptibility or resistance to pesticides but also for one or more steps in the sequence of reactions involved in breaking of diapause. Some of the enzyme systems in microsomes contain either a heme or other colored co-factor such as riboflavin as the prosthetic group.

Epoxidation of aldrin to dieldrin has been studied and found to occur with microsomal preparations obtained from the corn earworm, tobacco budworm, the codling moth, the pink bollworm, the European corn borer (Ostrinia nubilalis), and the red banded leaf roller (Argyrotaenia velutinana). (204, 207)

b. Protein synthesis studies. DL-Leucine- $l$ -C<sup>14</sup> has been employed to study the relative rates of in vivo incorporation of amino acids into proteins in the European corn borer under light regimens which induce diapause as well as those which facilitate growth and development. Treatment of the corn borers with cycloheximide (which influences the interaction of messenger RNA and susceptible ribosomes and/or affects methylation of RNA) also results in a delay in development which seems to stimulate diapause to some extent. A comparison of metabolism of materials such as leucine-C<sup>14</sup> between insects in which diapause has been induced or broken by a suitable photoperiod and insects treated with cycloheximide (or other similar material) is providing insight into the relevant site(s) of action of and the sequence(s) of biochemical events initiated by light. Others have shown that protein synthesis is affected by changing the concentrations of ammonium ions in the suspending medium. Injections of NH<sub>4</sub>Cl have been found to be toxic to European corn borers. In preliminary studies designed to lead to practical use of this finding, insects were immersed briefly in a variety of inorganic salt solutions in an attempt to upset their biochemistry or kill the insects. These inorganic salts included: (a) A saturated solution of calcium phosphate applied topically, which proved toxic to H. zea and H. virescens larvae and to adult boll weevils. (b) A mixture of KNO<sub>3</sub>(0.05-0.1M), NH<sub>4</sub>Cl(0.05-0.1M), and detergent was toxic to H. zea and H. virescens pupae. (207)

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AREA NO. 19 - FUNDAMENTALS OF INSECT STERILITY

USDA and Cooperative Programs

Location of Intramural Work	: Scientist Man-Years FY 1968 :					Total
	: Research Problem Area :					
	: 204 :	207	: 210	: 706	:	
North Dakota	: 2.6 :	3.2	: 0.9	: 2.0	:	8.7

## Problems and Objectives

The present control of insect pests is primarily dependent on chemical insecticides. However the development of resistance to many of these by insects and the hazards of contamination of food, forage and the environment by harmful residues indicates an urgent need for alternate methods of insect control. The success of the sterility method applied to the screw-worm and Mexican fruit fly problems proves the validity of the method. Basic research is needed on the genetics and physiology of reproduction of insect pests and on the effects of various types of sterilants and dosages on the various life stages.

Objectives of the research are to determine:

1. Most effective dosages and methods of application of radiation.
2. Effects of mutagenic chemicals on reproduction and heredity.
3. Cellular effects from exposure to chemical mutagens and radiation.
4. Genetics of selected economically important insects.

## Progress - USDA and Cooperative Program

### A. Radiation Sterilization of Insects (3.0 SMY)

#### 1. Radiobiology of Lepidoptera

Lepidoptera require high doses of radiation to effect sterility. The resultant sterile males are usually not as competitive as desired in their ability to reduce the population size when released into natural field populations. Methods to increase their competitiveness are being developed. It was previously found that the progeny from males receiving a substerilizing dose of radiation were completely sterile. In laboratory tests it was determined that by introducing substerile males into a normal population at ratios of 9 substerile males to every fertile male in the population, the next generation contains 60% sterile individuals. The reproductive potential of the next generation is reduced by fivefold as compared with that expected of a natural population. This method affords a more efficient means of population control. Furthermore, the inherited sterility persists for at least 5 generations when the progeny from the initially irradiated males are interbred. In practical field tests, the future population size should be decreased substantially and also the sterile individuals would be field-reared, which should be a distinct advantage over releasing laboratory-reared insects into a natural environment.

The primary reason completely sterile lepidopterous males fail to compete with normal males in reducing the reproductive potential of a population is their inability to transfer sperm. If a female does not receive an adequate supply of sperm or accessory fluid, a normal oviposition response is not elicited. The reasons behind this are presently being explored with the hope that more complete knowledge of the situation will lead to possible means of alleviating this detriment.

In addition, it has recently been found that the immature stages tolerate much higher doses of radiation than expected without lethality. Studies on differential radiosensitivity of various cell stages leading to sperm formation are being studied with the future possibility of either inducing sterility at these earlier stages or producing an aspermic male that may be competitive and also elicit a normal oviposition response from the female. (Multiple RPAs)

## 2. F<sub>1</sub> Sterility in the Tobacco Budworm

Studies have been conducted on the inherited sterility of the tobacco budworm, Heliothis virescens. In general, when male budworms are irradiated with substerilizing doses of 7.5, 15, and 22.5 kr, the following are observed: (1) The progeny from such crosses when outcrossed with normal moths are more sterile than the parents. (2) F<sub>1</sub> females mated to normal males and normal females mated with F<sub>1</sub> males laid fewer eggs than normal females mated to normal males. (3) The F<sub>1</sub> males did not mate as often nor transfer sperm as readily as the normal males. (4) The larval developmental time is longer for the F<sub>1</sub> individuals than the normal ones. (5) In the F<sub>1</sub> progeny, the sex ratio is altered in favor of the males.

When the original male parents were irradiated with 0 (control), 7.5, 15, and 22.5 kr and their F<sub>1</sub> progeny were studied, the F<sub>1</sub> males crossed to normal females gave egg hatches of 79.6, 33.5, 5.3, and 2.2 for the respective radiation doses, and the F<sub>1</sub> females crossed to normal males gave egg hatches of 61.3, 29.5, 9.4, and 1.0. Thus, the F<sub>1</sub> were more sterile than the irradiated parents. At 15 kr the frequency distribution of egg hatch is strongly skewed towards sterility. Although some pairs show high egg hatch, the percent egg hatch is 5% or lower in 80% of the crosses. Furthermore, the percent egg hatch does not indicate the full reduction in reproductive potential because the fecundity of the F<sub>1</sub> outcrosses is also reduced. The average number of eggs per female for the 4 radiation treatments was 598.1, 549.3, 398.6, and 272.0 when F<sub>1</sub> males were crossed to normal females and 696.4, 432.0, 336.2, and 216.0 when F<sub>1</sub> females were crossed with normal males.

In assessing mating ability, 3 criteria were used: (1) whether mating occurred; (2) how frequently mating occurred; and (3) whether sperm was transferred. The mating capacity of the F<sub>1</sub> males was reduced at all doses. At 7.5, 15, and 22.5 kr, only 80, 70, and 67%, respectively, of control mating was observed. Of the males that mated, the ability to transfer sperm was also reduced. At 7.5, 15, and 22.5 krads, only 55, 52, and 27% of the F<sub>1</sub> males that mated transferred sperm. For the control, 88% of the males that mated were capable of transferring sperm.

Larvae whose male parent received 15 or 22.5 kr of gamma radiation took 2 to 2.5 days longer to develop than did the control or larvae from male parents irradiated at 7.5 kr. This was true for both F<sub>1</sub> males and F<sub>1</sub> females. There was no significant difference in the pupal developmental time of any treatment.

At the higher 2 doses, from 2 to 3 males per female were recovered. In comparison, the sex ratio of the control and of F<sub>1</sub> males and females from male parents irradiated with 7.5 kr was nearly 1:1 (207)

### 3. Pieris Irradiation Studies

In cooperation with the Biological Control Laboratory, Columbia, Mo., radiation-sterilization studies were conducted on the imported cabbage worm, Pieris rapae L. Male butterflies required 35 kr to induce 95% sterility and about 48 kr for complete sterility. Females required 21 kr for complete sterility. No difference was found in the average number of eggs per female laid by control females or those mated to irradiated males. However, irradiation of the female caused reduced oviposition. Lifespans of irradiated females were not reduced by irradiation although the males irradiated with doses of 21 to 35 kr showed a 30% reduction in lifespan. The percent of females mated and the average number of spermatophores per female were not affected by irradiation of either sex. (204)

### 4. Irradiation of Heliothis

In earlier radiation studies with the tobacco budworm, Heliothis virescens (F.), reduced oviposition from females mated to sterilized males caused concern about possible radiation damage to the mating and sperm transferring ability of the male. We recently found that untreated male tobacco budworms occasionally transfer a spermatophore that does not contain sperm. However, tobacco budworms sterilized by 45 kr had a significantly greater number of failures to transfer sperm. Females that received a spermatophore but had no sperm in their spermathecae produced the same numbers of eggs as virgin females, or about 20% as many eggs as females with sperm in their spermathecae. Females with sperm in their spermathecae laid normal numbers of eggs, whether the sperm came from irradiated or untreated males. The failure of the male to transfer sperm during mating occurred because sperm were not incorporated into the spermatophore.

Therefore, eradication programs based on the release of sterile lepidopterous males must provide for the reduced ability of the sterile male to inseminate females. (207)

### 5. Radiation Sterilization of the Boll Weevil

Attempts were made to induce complete and permanent sexual sterility in boll weevils. Certain treatments with X-rays or gamma-rays and apholate resulted in moderate mortality and complete initial sterility. However, fertility was eventually recovered, and mating competitiveness was reduced.

Thermal neutrons failed to sterilize boll weevils at sublethal doses. Fast neutrons significantly reduced fertility of treated males and fecundity of treated females at 1 kr. Doses above 1.2 kr caused high mortality, and 2 kr were required to induce sterility in all sperm and to destroy all spermatogonia.

Irradiation of either sex with 1 kr followed after 2 days of immersion in 1% apholate resulted in almost complete sterility; however, the spermatogenic cycle was re-established in some individuals at 4 weeks after treatment. Mating competitiveness of weevils treated with fast neutrons and apholate was reduced less than by comparable treatments by gamma-rays and apholate. (207)

#### 6. Irradiation of House Flies -- Effect on the Utilization of Pupal Fat Body

The adult house fly emerges with a large supply of nutrients contained in pupal fat body cells which are found in the haemolymph. These pupal fat body cells disappear within a few days after emergence and several workers have suggested that the nutrient products are used in ovarian development. Our studies with male and female flies irradiated as either pupae or adults with the minimum doses of radiation required to prevent ovarian development indicate that the utilization of pupal fat body is the same in both sexes. When young adults are irradiated, the rate of utilization is not different than in untreated flies. However, when pupae are irradiated, the utilization rate is significantly slowed. In all cases, the irradiated females do not develop ovaries. It is obvious that the utilization of pupal fat body is not dependent on ovarian development, or even on the sex of the fly. (706)

#### B. Chemosterilants and Mutagens Affecting Insect Reproduction and Heredity (2.5 SMY)

##### 1. Search for a Sperm Inactivating Chemical in the House Fly

Seventeen diverse compounds were tested to determine whether sterility could be produced in house flies. All compounds tested were non-aziridine chemosterilants and, according to their chemical properties, were either injected, fed, or topically applied to male house flies. In those instances where total or partial sterility was obtained, further studies were conducted to determine whether the sterility produced was due to the induction of dominant lethals or sperm inactivation.

Of all the chemicals tested, 1,3-Propanediol methane sulfonate (ENT-51904) was the most efficient sterilant. This chemical induced sterility at relatively low doses with negligible mortality. Cytological studies of eggs fertilized with sperm treated with ENT-51904 revealed that it produces its sterilizing effect in the male house fly by inducing chromosomal damage in sperm and not by inactivation.

Among the other chemicals tested were several organotin compounds. Although all were fairly toxic and gave high mortality, hydroxytriphenyltin (ENT-28009), appears to induce a latent type of sperm inactivation. The partial sterility of females fertilized by males topically treated with ENT-28009 was increased by eliminating available oviposition sites for 7 days following copulation. Preliminary tests show that egg hatchability is reduced 45% by compelling females to store their complement of sperm before

use. Additional studies are planned to positively confirm this phenomenon and to further investigate the mechanism of sperm inactivation. (706)

## 2. Cytogenetic Studies of Action of Chemosterilants in the House Fly

Studies were conducted with the house fly to delineate the mode of action of tepa, hempa, and 4 phosphine aziridiny1 analogs of tepa (ENT-50990, 50991, 50716, 50107). In these studies it was attempted to determine whether males treated with these compounds transferred sperm in normal amounts during mating, whether these sperm were utilized by the females and retained motility, whether the pattern of polyspermy in the fertilized eggs was similar to that encountered in the controls, and finally what prevented the eggs from hatching after sterilization with chemosterilant-treated sperm.

The treatment of male house flies with sterilizing doses of tepa, hempa, or the 4 analogs before mating with untreated females did not reduce the number of eggs that were fertilized. However, the incidence of polyspermy was significantly lower in eggs fertilized with sperm from males treated with tepa, hempa, and 1 analog. Thus, the possible inactivation of sperm by the chemosterilants would not be as evident in a polyspermic insect as in a monospermic species. The cytological examination of the eggs for chromosome abnormalities produced by these 6 chemicals revealed that all test chemicals resulted in the formation of chromosome bridges and fragments during early embryogenesis. These genetic abnormalities led to mitotic arrest and other developmental abnormalities and caused cessation of embryonic development. Although the chemicals differ structurally, all eventually produced similar cytogenetic damage, but there was evidence of some significant differences in the time of expression and kind of chromosome aberrations produced by the various chemicals. (706)

## 3. Chemosterilization of Heliothis

Several chemosterilant compounds were previously tested by topical application to adult tobacco budworms, Heliothis virescens (F.). During the last year, 16 chemosterilants (aziridines, methanesulfonates, a nitrogen mustard, and phosphoramidate compounds) were evaluated by feeding them to adults of the tobacco budworm, Heliothis virescens (F.). Promising chemosterilants were found only among the aziridines, a number of which sterilized both sexes. Either sex was sterilized by 100 µg/moth or less of the following compounds: tepa, metepa, tretamine, and ENT Nos. 51254, 50716, and 25294.

The doses of the most promising aziridines required to sterilize the males were about half those required to sterilize females. Mating and lifespan appeared least affected by tretamine and ENT-50716. Thus, chemosterilization of the tobacco budworm can best be accomplished by aziridine compounds. Our studies indicate that either topical application or feeding may be used to administer the chemosterilant. (207)

C. Cellular Effects in Insects Resulting from Exposure to Chemical Mutagens or Radiation (1.5 SMY)

1. Ultrastructure of Insect Reproductive Cells and Chromosomes in Relation to Radiosensitivity

Several electron microscope studies on the ultrastructure of insect reproductive tissues are underway. In the cabbage looper, the ultrastructure of apyrene and eupyrene sperm together with their surrounding nurse cells is currently under investigation. It has already been shown that the anucleate apyrene sperm are released from the testes simultaneously with the eupyrene forms and that both types are transferred to the females during mating. Observations on the ultrastructure of seminal vesicles in the cabbage looper, tobacco budworm, boll weevil, milkweed bug, and black blowfly, Phormia regina, have revealed many similarities, including evidence of a high level of synthetic activity suggesting that the vesicle cells contribute material to the semen.

Preliminary observations have been made in a proposed study of centromeric regions and their spindle attachments in chromosomes of the cabbage looper, milkweed bug, and the house fly. It is hoped that study will give information concerning the wide differences in radiosensitivity of the 3 species. (204)

2. Comparative Sensitivity of Mature Sperm and Gonial Cells as Related to Permanence of Sterility

Work was completed on a comparative study of spermatogonial death and the induction of dominant lethal mutations in mature sperm in 3 species of Diptera, Musca domestica, Phormia regina, and Cochliomyia macellaria. Radiation dosages required to produce dominant lethal mutations in all the mature sperm were fairly similar for all species. However, wide differences were found in dosages required to destroy all primary spermatogonia in the 3 species and thus prevent eventual recovery of fertility. Gonial cells in C. macellaria were distinctly more radioresistant than those of the house fly. (210, 706)

3. Permanence of Chemosterilant Induced Sterility in House Flies

The effects of 12 chemosterilants were studied to determine whether the sterility induced in house fly males would be permanent or temporary. The experiments were designed to determine whether treatments that induced dominant lethal mutations in the mature sperm would also destroy the gonial cells of the treated male and thus prevent recovery of fertility after treatment. The 12 chemosterilants studied could be classified into 3 groups on the basis of comparison between effects on mature sperm and gonial cells. Chemosterilants such as tretamine, tepa, hempa, apholate, and ENT-50172 were very effective in killing gonial cells at the minimum doses required to induce dominant lethal mutations in the mature sperm. Other chemicals, such as diepoxybutane, ENT-51909, nitrogen mustard, and ENT-50838 permitted

a high degree of recovery of spermatogenic activity in the testes of males at doses that induced dominant lethal mutations in all the mature sperm. Consequently, recovery of fertility from a completely sterilizing treatment appears very likely with these chemicals. On the other hand, methanesulfonate chemosterilants, such as ENT-51799, ENT-51904, and EMS, had virtually no effect on the gonial cells and complete recovery of spermatogenic activity in the testes was observed even at doses that were more than that needed to induce dominant lethal mutations in all the mature sperm. Recovery of fertility after an initial sterile period is expected after these treatments. In fact, studies with males injected with ENT-51904 showed that although the males were completely sterile in the first 2-3 matings, they completely recovered their fertility by the 7th and 8th matings. (706)

#### D. Genetics of Selected Economically Important Insects (1.7 SMY)

##### 1. Genetics of the House Fly

The linkage group-karyotype correlation that was recently established by the cytological analysis of X-ray induced autosome-autosome translocations has been confirmed by the cytological analysis of Y-autosome translocations. The previous assignment of 5 linkage groups to specific chromosomes has been definitely confirmed.

Tests with house flies heterozygous for different chromosome translocations are being continued to determine which ones are the best combinations for decreasing fertility. All of the tests thus far have given the theoretically expected decreased fertility and, therefore, show promise for population suppression of insect pests.

The use of meiotic-drive factors for insect population suppression are being studied. Meiotic drive produces a segregation distortion from the expected types of progeny. Deleterious genes could be forced into a population via these meiotic-drive (segregation distortion) factors. We have treated house flies with X-rays to induce meiotic-drive factors. Examination of F<sub>2</sub> progeny from individual F<sub>1</sub> flies has produced 18 cases of segregation distortion which is perpetuated generation after generation. A search for further meiotic-drive factors will be continued, and the factors found thus far will be tested in population cages for their possible application to insect population suppression.

A search has also been undertaken for cases of hybrid sterility or cytoplasmic incompatibility in the house fly with strains collected in different geographical locations. Flies from the United States, Italy, Japan, Union of South Africa, and Australia have been intercrossed in appropriate ways. No classical cases of hybrid sterility or cytoplasmic incompatibility have been found thus far. However, cases of reduced reproductive potential have been found, and a case of sex-ratio distortion has been found where 95% of the progeny of such crosses are males. This

sex-ratio distortion persists for many generations and can be manipulated at will. These strains are promising as possible tools for insect control. Further cytological and genetic studies will be made to ascertain the causes for these distortions and further tests are planned with strains from other locations.

Mutant house fly strains have been rated against a wild-type strain for ability to survive and reproduce in population cages. To date, no mutant has compared favorably with the wild-type fly. Such recessive mutants could be deleterious or even lethal when expressed in nature. Introduction of such genetic factors will be made through the utilization of heterozygous carriers. Future cage tests call for the release of individuals heterozygous for several mutants into wild-type populations. Competitive mating tests and tests of penetrance are also planned.

Studies have been initiated in order to isolate autosomal inversions in the house fly. Results to date with chromosome 3 have been negative. Inversions would be useful for the study of recessive lethal factors and could be employed for the isolation of various isogenic chromosomal segments. Inversions might also be utilized to localize genes on chromosomes. Such localization would be valuable in pinpointing insecticide resistance factors. Further, it is possible that pericentric inversions could be utilized for control purposes through reduction of female fertility. Such reduction would necessarily involve long pericentric inversions in which crossing over takes place. (706)

#### E. Changes in Insect Tropisms with Sterilizing Treatments

Studies were carried out to determine if gamma irradiation of 5-day-old pupae would alter the response of adult house flies to temperature and humidity gradients and to oxygen uptake. Comparisons were made between males and females of various ages irradiated in the pupal stage and unirradiated controls. Computer analysis of data indicated that there were some significant differences between the sexes and between flies of various ages in their responses to these 3 indices of physiological response or humidity preference between irradiated and nonirradiated male flies. Oxygen requirements for both males and females were more markedly affected by age than by radiation. (706)

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